Wastewater profiling for community-wide human exposure assessment from environmental endocrine disrupting chemicals in personal care and consumer products

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Wastewater profiling for community-wide human exposure assessment from environmental endocrine disrupting chemicals in personal care and consumer products

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ABBREVIATIONS

EDCs (Endocrine Disrupting Chemicals)

TDI (Tolerable Daily Intake)

EFSA (European Food Safety Agency)

LC (Liquid Chromatography)

MS (Mass spectrometry)

LC-MS (Liquid Chromatography coupled with Mass spectrometry)

HRMS (High Resolution Mass Spectrometry)

SPE (Solid Phase Extraction)

MAE (Microwave Assisted Extraction)

HLM (Human Liver Microsomes)

S9 (Human S9 fraction)

WBE (Wastewater Based Epidemiology)

WWTP (Wastewater Treatment Plant)

SPM (Solid Particulate Matter)

PCP (Personal Care Product)

BP-1 (benzophenone-1)

BP-2 (benzophenone-2)

4,4'-DHBP (4,4'-dihydroxybenzophenone)

4-BenzPh (4-benzylphenol)

HO (homosalate)

OC (octocrylene)

3-BC (3-benzylidene camphor)

4-MBC (3-(4-Methylbenzylidene) camphor)

EHMC (ethylhexylmethoxycinnamate)

BPA (bisphenol A)

BP-3 (benzophenone-3)

BDE209 (Bis-(pentabromophenyl)ether)

HBCD (Hexabromocyclododecane)

TBBPA (Tetrabromo bisphenol A)

DNB (Di-n-butyl- phthalate)

BADGE (Bisphenol A diglycidyl ether)

DEHP (Bis(2-ethylhexyl) phthalate)

MEHP (mono (2-ethyl-5- hydroxyhexyl) phthalate)

MECPP (mono (2-ethyl-5-carboxypentyl) phthalate)

MEOHP (mono (2-ethyl-5-oxohexyl) phthalate)

PFOA (Perfluorooctanoic acid)

PCMC (4-chloro-3-methylphenol)

Chloroxylenol (4-Cl-3,5-dimethylphenol)

Padimate O (2-Ethylhexyl 4-(dimethylamino)benzoate)

PBSA, Ensulizole (Phenylbenzimidazolesulfonic acid)

MeOH (Methanol)

FA (Formic acid)

HDPE (High-density polyethylene)

IDL (instrumental detection limit)

IQL (instrumental detection limit)

MDL (Method detection limit)

MQL (Method quantification limit)

RSD (Relative standard deviation)

IS (labelled internal standard)

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1 General introduction and objectives of the thesis

1.1 Overview

The aim of the thesis is to develop a tool that harnesses the great potential of wastewater analysis to enable real time estimation of public exposure to well-known and newly identified chemicals. A real time profiling of community health and lifestyle has been highlighted as an emergent necessity to establishing public health decision making strategies. For many years population health status and habits have been investigated through methodologies such as general population surveys, questionnaires and surveillance studies that provided a comprehensive picture of the community only after years making it difficult to employ effective and successful policies. To overcome the above limitations a new monitoring programme needed to be undertaken. A real-time measurement of human health indicators can be achieved through analysing wastewater. Wastewater fingerprinting for obtaining information at community level population is a pioneering approach which, although still in infancy, is currently used as complementary tool in the assessment of populations health and lifestyle. This approach assumes that after internal exposure to chemicals (e.g. EDCs), human metabolic products (urinary biomarkers) are collected and pooled by the wastewater systems and are transported to wastewater treatment plants (WWTPs), providing evidence of the population exposure. Considering that WWTPs serve well-defined populations (in terms of numbers), estimation of both internal and external exposure to chemicals (e.g. pharmaceuticals usage, drugs consumption, EDCs exposure) in a given period (e.g. day, week) can be made based on analysis of wastewater (usually with sensitive and selective mass spectrometry techniques) after considering human metabolism patterns (and possible transformation in WWTPs) of target analytes. In the longer term the tool will allow for an evaluation of the relevance between exposure and effects permitting prompter and more efficient strategies to lower the general risk associated to the exposure to selected chemicals. In order to achieve that four objectives were identified.

1.2 Aim of the research programme

The aim for the research programme is to better understand exposure to endocrine disrupting chemicals used in personal care and consumer products.

To achieve that a list of compounds of interest consisting in poorly investigated chemicals not intended for human consumption will be identified (objective 1). Since the metabolism of the selected compounds has been scarcely investigated a new tool to identify metabolites suitable as biomarkers of exposure combining WBE and *in vitro* techniques will be developed (objective 2). Once new metabolites suitable as biomarkers of exposure will be identified applying the newly developed tool (objective 3) a new analytical method will be developed and validated to investigate the presence of the selected compounds, biomarkers and newly discovered potentially toxic chemicals (along with their metabolites) in different environmental matrices to understand internal and external exposure (objective 4).

1.3 Objective 1

Objective one is to identify a list of EDCs (or suspected to be) used in personal care and consumer products not intended for consumption whose exposure patterns and long-term effect are mostly unknown. The exposure to the commonly used plasticizer bisphenol A via analysis of its metabolite bisphenol A sulphate will be proposed as case study to highlight the great potential of wastewater based epidemiology (WBE) approach.

1.4 Objective 2

The first objective of the thesis is to develop methodology enabling identification of biomarkers of exposure via wastewater fingerprinting. The analysis of metabolic biomarkers of target chemicals is crucial to distinguish between internal exposure and direct disposal, since many sources contribute to chemicals being discharged into wastewater. As there were many gaps in literature with regards to the metabolism of many of the selected compounds denoting many difficulties in selecting biomarkers of exposure to undertake population studies, a key aspect of the project will be the development of a new tool for identifying new metabolites suitable as biomarkers of exposure for wastewater analysis. In order to identify human specific metabolites excreted with urine and consequently present in wastewater, and to assess exposure to a wide range of chemicals, a new systematic workflow involving the employment of in vitro techniques along with urine and wastewater analysis will be developed.

1.5 Objective 3

The third objective of the thesis is to identify biomarkers of exposure to EDCs in personal care products. The developed method which combines *in vitro* incubation techniques with urine and wastewater analysis will be applied to commonly used compounds for which there is scarce knowledge about their metabolism. The results will permit the selection of suitable candidate biomarkers of human exposure for possible application in wastewater-based epidemiology.

1.6 Objective 4

The fourth objective is to develop a comprehensive framework for quantitative analysis of EDCs and retrospective identification of metabolites. A multi-residue and sensitive methodology for the targeted analysis of chemically diverse suspected and proven EDCs in sewage and environmental matrices will be developed and validated. Liquid chromatography coupled to high resolution mass spectrometry will be used to permit the identification of metabolites and biodegradation products and eventually retrospective analysis. The developed and validated method will be applied to pooled environmental samples to assess the presence of the potentially toxic and persistent chemicals. Finally, once EDCs will have been detected and quantified in all investigated environmental matrices the study will focus on the identification of new and known biomarkers of exposure for specific EDCs in influent wastewater in order to find out any possible correlations with exposure.

This declaration concerns the article entitled:	
Water fingerprinting for community-wide exposure assessment to endocrine disrupting chemicals in household products: exposure to bisphenol A	
Publication status	
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Publication details	Environmental Science & Technology
Candidate's contribution to the paper	**Formulation of ideas:** Luigi Lopardo and Barbara Kasprzyk-Hordern planned and designed the study. organised the collection of wastewater samples. **Design of methodology:** Luigi Lopardo and Barbara Kasprzyk-Hordern organised the collection of wastewater samples. **Experimental work:** Luigi Lopardo prepared and analysed the samples and interpreted the results. **Presentation of data in journal format:** Luigi Lopardo and Barbara Kasprzyk-Hordern prepared the manuscript, which was critically revised by all co-authors
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.
Signed	**Date**

2 Water fingerprinting for community-wide exposure assessment to endocrine disrupting chemicals in household products: exposure to bisphenol A

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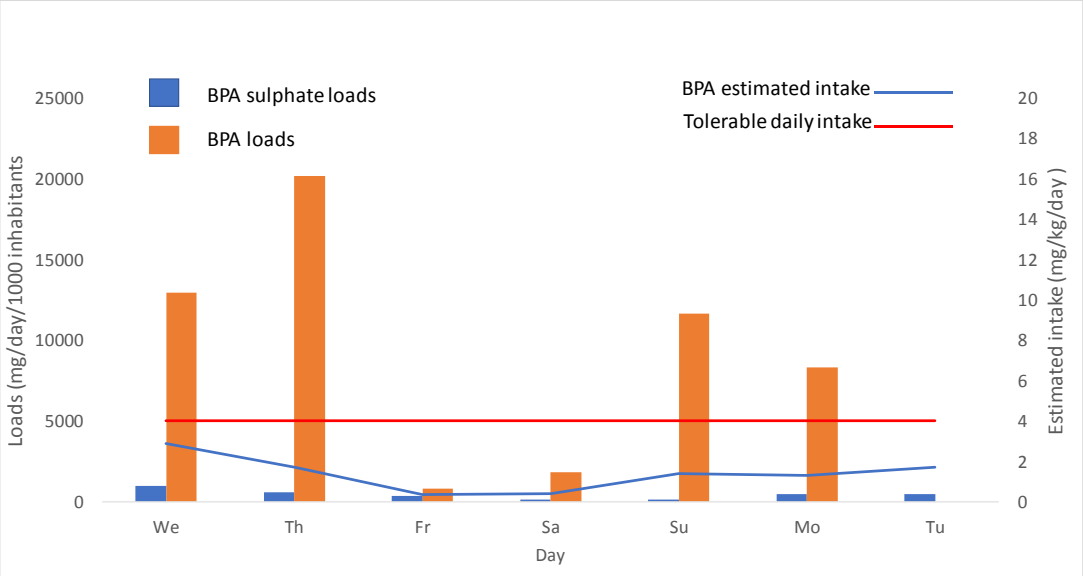
2.1 Graphical abstract



Keywords: water fingerprinting, endocrine disruptors, bisphenol A, epidemiology, exposure, WBE

2.2 Abstract

Background Molecular epidemiology in human biomonitoring allows for verification of public exposure to chemical substances. Unfortunately, due to logistical difficulties and high cost, it evaluates only small study groups and as a result does not provide comprehensive large-scale community-wide exposure data. Wastewater fingerprinting utilising metabolic biomarkers of exposure that are excreted collectively by studied population into urine and ultimately into community's wastewater, provides a timely alternative to traditional approaches.

Objectives This study aimed to provide comprehensive spatiotemporal community-wide exposure to bisphenol A (BPA, including BPA intake) using wastewater fingerprinting.

Methods Wastewater fingerprinting was undertaken using high resolution mass spectrometry retrospective data mining of characteristic BPA human metabolism marker (Bisphenol A sulphate), applied to a large geographical area of 2,000 km^2 and a population of ~1.5 million accounting for >75% of the overall population in the studied catchment.

Conclusions Community-wide BPA intake was found to be consistently below the tolerable daily intake (TDI) level set by the European food safety agency (EFSA) suggesting overall low exposure, with an exception of two locations, where higher BPA sulphate loads corresponding to higher intakes (above the TDI threshold) were observed. Characteristic temporal variations of BPA intake were noted in most studied wastewater treatment plants (WWTPs) with the lowest intake occurring during weekends and the highest during weekdays. Further work needs to be undertaken to fully understand sources of BPA and BPA sulphate that might be contributing to the biomarker concentration levels in the studied catchment.

2.3 Introduction

Real time assessment of communities' health status is crucial for optimal decision-making strategies and resources investment for the prevention, control and mitigation of exposure risks aiming at improving populations health and well-being. The standard of living has improved but at the same time the incidence of illness such as cancer or obesity is increasing with very little understanding of their causes. One potential reason could be an augmented human exposure to a wide range of man-made environmental chemicals in e.g. household chemicals such as personal care products (PCPs). By comparing community levels of environmental stressors with observed health effects, conclusions could be drawn as to whether elevated levels of certain chemicals could be linked with particular diseases. For many years, epidemiological information about lifestyle habits, public health and wellbeing has been gathered via methodologies such as general population surveys and other surveillance tools that relied on patient's experience and sense of wellbeing (not necessarily objective). These approaches are prone to self-reporting bias and require long time for data collection and analysis, which makes active prevention of exposure risks by policy makers extremely difficult. Therefore it is crucial to undertake more timely and efficient approaches to identify cause-effect linkages between environmental stressors and communities health status (Daughton 2012b), with particular focus on the effects that complex mixtures might have on human population (particularly with regard to reproductive health)(Kortenkamp et al. 2011). These approaches monitor biological responses rather than diseases in human populations through the usage of biomarkers.

Biomarkers (the biological endpoints) are quantifiable entities defining physiological or pathological biochemical processes occurring in the body e.g. unusual chemical metabolites, *in vivo* genetic changes, and alterations in gene expression, proteins and cell-based markers. The use of biomarkers in epidemiological research provides invaluable and objective information about patient's health and disease status, even if not detected via classical endpoints when variations are undetectable and/or without immediate effect on health, and therefore do not necessarily correlate with a patient's experience and do not correspond to patients' clinical state. Hence the measurement of biomarkers allows a snap-shot of health and disease of a given individual or population, its state or increased risk for disease, which are vital to the field of preventative medicine. For these reasons biomarkers are likely to substitute classical

endpoints such as disease incidence or mortality and have positive impact on public health (Bonassi and Au 2002). However, a limitation of molecular epidemiology, due to logistical difficulties and high cost, is the restricted size of study groups and inability to gather comprehensive information on the complexity of combined (and cumulative) spatial and temporal exposure to mixtures of endocrine disrupting chemicals (EDCs) and their effects (Bonassi and Au 2002). Therefore the community lacks robust measures that can be used to gather real-time information on community-wide health.

2.3.1 Wastewater-based epidemiology for public health assessment

The advancement of the new field of wastewater-based epidemiology (WBE) and its unique approach towards retrieving epidemiological information from wastewater via the analysis of human biomarkers might overcome the above limitations and could provide for the first time real-time measurements of community-wide exposure to household EDCs. This highly innovative approach has been pioneered by the SCORE (www.score-cost.eu) and Sewprof groups (www.sewprof-itn.eu), and, although still in its infancy, is currently used to determine community-wide illicit drug use (Ort et al. 2014; Thomas et al. 2012). A significant study carried out in 2013 outlines the great potential of this approach. In this work involving 23 cities in 11 different European countries, samples of wastewater from wastewater treatment plants (WWTPs) serving a total of over 24 million people were analysed permitting the monitoring of consumption patterns of cocaine, amphetamine, methamphetamine, cannabis and MDMA with cocaine found in almost every city investigated while the consumption of other drugs of abuse varied in different European regions (Ort et al. 2014). Another example is represented by the study carried out by Castrignanò et al. in 2018 in which enantiomeric profiling of chiral illicit drugs in wastewater serving 4.9 million people in eight European cities was undertaken to verify potency of drugs use and to estimate the extent of consumption vs direct disposal of unused drugs to the sewerage system (Castrignanò et al. 2018).

The key concept of this approach is that human biotransformation products of both endogenous or exogenous compounds resulting from exposure to xenobiotic agents such as drugs, food toxicants and pollutants are collected and pooled by the wastewater system, and are transported to WWTPs, providing evidence of the extent of the

exposure for the population (Fig. 1). As WWTPs serve well-defined populations (in terms of numbers), estimation of exposure to chemicals (e.g. drug consumption) in a given period can be made based on analysis of wastewater (usually with sensitive and selective mass spectrometry techniques) after taking into account human metabolism patterns (and possible transformation in WWTPs) of target analytes. Earlier work with therapeutic drugs has demonstrated the close correspondence between the known amounts consumed by the population and the amounts estimated from concentrations of metabolic drug residues in wastewater (Heberer and Feldmann 2005; Kasprzyk-Hordern et al. 2009) or in surface waters (ter Laak et al. 2010).

Table 2.1 gathers examples of WBE application in public health and lifestyle assessment. For example, Ryu et al. (Ryu et al. 2016b) reported a Europe-wide monitoring of an oxidative stress biomarker, 8-iso-PGF2α and found that increased levels of 8-iso-PGF2α were observed at the inner-city level correlating with the degree of urbanization and levels of nicotine use. Rousis et al. (Rousis et al. 2017b) studied community-wide exposure to pyrethroid pesticides in Italian cities. Gracia Lor et al. (Gracia-Lor et al. 2017) undertook Europe-wide profiling of caffeine use. Lopardo et al. (Lopardo et al. 2017) identified new biomarkers of internal exposure to endocrine disruptors. González-Mariño et al. (González-Mariño et al. 2017) investigated community-wide exposure to phthalate plasticizers in Spain.

Table 2.1 WBE for public health and lifestyle assessment

Compounds	Biomarker	Health status - comments	References
Illicit and abused drugs	Drugs and urinary metabolites Enantiomeric enrichment	Lifestyle	(Andrés-Costa et al. 2017; Castrignanò et al. 2017, 2018; Jones et al. 2014; Kasprzyk-Hordern et al. 2008; van Nuijs et al. 2011)
Alcohol	Ethyl sulphate, Isoxanthohumol, Resveratrol Metabolites	Lifestyle	(Reid et al. 2011; Ryu et al. 2016a)
Caffeine	1-methylxanthine, 7-methylxanthine	Lifestyle/population biomarker	(Gracia-Lor et al. 2017; Senta et al. 2015)

Tobacco	cotinine, tobacco specific nitrosamines, menthol, 8-iso-prostaglandin F2α	Lifestyle	(Lai et al. 2017; Ryu et al. 2016b)
Pharmaceuticals	Specific pharmaceuticals and their metabolites Enantiomeric enrichment	Health	(Camacho-Muñoz et al. 2016; Jones et al. 2014; Kasprzyk-Hordern et al. 2008; Petrie et al. 2015)
Endocrine disruptors	Urinary metabolites	Health	(Lopardo et al. 2017, 2018)
Pesticides	Specific pesticides and their metabolites Enantiomeric enrichment	Health	(Rousis et al. 2016, 2017a, 2017b)
Phthalates	Urinary metabolites	Health	(González-Mariño et al. 2017)
Oxidative stress	8-iso-prostaglandin F2alpha	Health	(Ryu et al. 2015)
Cancer	mtDNA	Health	(Yang et al. 2017)

2.3.2 WBE as epidemiology tool for human exposure assessment from EDCs in household products

EDCs are exogenous chemicals with the potential to interfere with the hormonal regulation, hence with the endocrine system, consequently affecting health and reproduction in animals and humans. In addition to developmental and reproductive effects, their potential for contributing to metabolic disorders such as obesity is drawing more and more attention (Casals-Casas and Desvergne 2011). In the recent EU document *'State of the art assessment of endocrine disrupters'* (Kortenkamp et al. 2011) there is an urgent call for new approaches to establish further evidence for humans' exposure to EDCs, especially those chemicals which are still not regulated (such as many suspected EDCs in personal care and consumer products). Regulatory decisions about endocrine disruptors will have to rely on weight-of-evidence procedures which are yet to be established (Kortenkamp et al. 2011). It is therefore important to develop new tools which will allow for long term real-time monitoring of collective community-wide exposure to and effects from EDCs.

WBE has a potential to overcome some of the above difficulties and is able to assess both internal and external combined exposure to EDCs. In the longer term the tool will

allow for an evaluation of the relevance between exposure and effects due to the utilisation of both biomarkers of exposure (EDCs and their characteristic metabolites) and biomarkers of effects (e.g. DNA or protein adducts, gene mutations).

Exposure assessment can be undertaken through the analysis of both parent EDCs and their metabolites in wastewater and in the environment. There are several points of verification. For example, the presence of characteristic metabolites in wastewater is an indication that EDCs have found their way into the body (internal exposure resulting from e.g. consumption of EDCs with food, accidental ingestion due to EDCs absorbed onto indoor dust, or absorption through skin of EDCs used in PCPs) (Fig 1).

The EDCs to which we are exposed to in our households are chemicals in personal care and consumer products, high volume environmental stressors with suspected or proven endocrine disruption actions (and with environmental fate and toxicological effects requiring additional study and evaluation). The list includes (but is not limited to) several man-made chemicals: plasticizers (e.g. phthalates, bisphenol A), UV filters (e.g. 4-methyl benzylidene camphor, octocrylene), surfactants (e.g. alkyphenols), preservatives (e.g. parabens), pesticides (e.g. vinclozolin); fragrances (e.g. musks), flame retardants (e.g. tetrabromobisphenol A), antimicrobials (e.g. triclosan and trichlorocarban). For most of these compounds, mainly non-regulated pollutants, there is no epidemiological exposure data (e.g. parabens (Kortenkamp et al. 2011)).

2.3.3 Metabolic biomarker section critical to public exposure estimation via water fingerprinting

The crucial step in the evaluation of public exposure to chemicals via WBE is the selection of suitable biomarkers of exposure, which must meet several sets of criteria. Gracia-Lor et al. highlighted the main requirements of a biomarker of exposure (i.e. high excretion rate in urine, detectability and stability in wastewater (Gracia-Lor et al. 2016)), which allow the distinction between internal exposure and direct disposal since many sources contribute to chemicals discharged into wastewater (Figure 2.1). As opposed to many compounds listed in Table 2.1, the EDCs investigated in this study are not meant for human consumption, hence, over the past years there has been little interest in their human metabolism patterns. Lack of characteristic metabolic biomarkers of EDCs evidencing internal exposure translates to major difficulties when attempting to apply WBE. The limited number of metabolic biomarkers known is

presented in Table S 2.1. However, given their extensive use in personal care and consumer products direct dermal exposure (amongst others) cannot be neglected and a deeper understanding of toxicokinetic processes (i.e. metabolism) is critical. An example of a work aiming to aid the identification of metabolic biomarkers of exposure to EDCs is the study carried out by Lopardo et al. (Lopardo et al. 2017). In this study a new analytical framework based on combining *in vitro* techniques with urine and wastewater analysis was developed (Figure 2.1). The observation of specific human metabolites (identified after *in vitro* experiments) of the antimicrobial 4-chloro-3-methylphenol in urine and wastewater proved that there is a critical need for expanding the list of biomarkers of exposure as the exposure to chemicals not intended for human consumption needs to be re-evaluated. In another study by Lopardo et al. (Lopardo et al. 2018) eight additional EDCs with unknown metabolic pathways (benzophenone-1 (BP-1); benzophenone-2 (BP-2); 4,4'-dihydroxybenzophenone (4,4'-DHBP); 4-benzylphenol (4-BenzPh); homosalate (HO); octocrylene (OC); 3-benzylidene camphor (3-BC); ethylhexylmethoxycinnamate (EHMC)) and two EDCs with known metabolism (bisphenol A (BPA) and benzophenone-3 (BP-3)) were tested. As a result of this study five metabolic biomarkers were identified (2-hydroxy-benzoic acid, 2-cyano-3,3-diphenyl-2-propenoic acid, 4-benzyl phenol sulphate, 4-benzyl phenol hydroxylated and sulphated and 4-benzylphenol-bi-hydroxylated and sulphated). The presence of metabolic biotransformation products of studied UV filters was confirmed in wastewater and this provides evidence for internal exposure of studied populations to these chemicals and needs to be investigated further.

This is the first study aimed to estimate community-wide exposure to BPA (including BPA intake) using wastewater fingerprinting. This study covers a large geographical area of 2,000 km^2 (including several rural and urban settlement) and a population of ~1.5 million accounting for >75% of the overall population in the studied catchment.

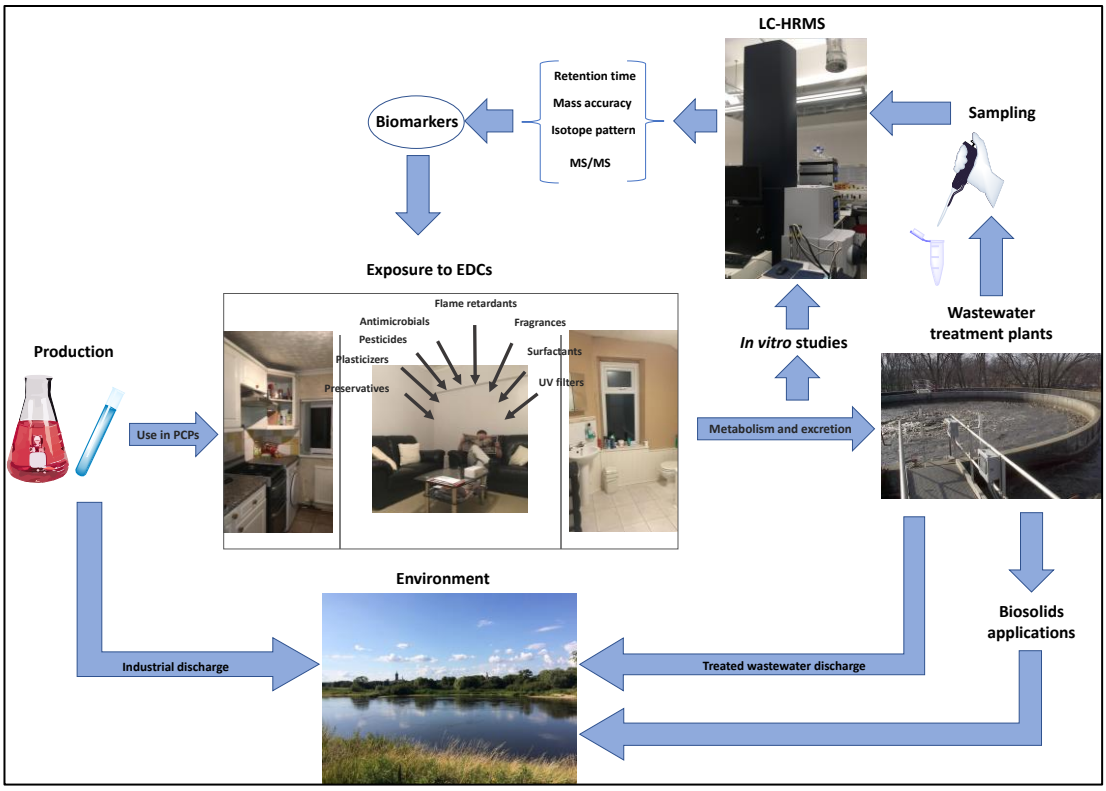


Figure 2.1 Schematic representation of exposure to EDCs in the household environment

2.4 Experimental section

2.4.1 Materials and chemicals

Bisphenol A sulphate (BPA sulphate, CAS 847696-37-1) was purchased from Toronto Research chemicals (TRC, Canada) (Table S 2.2). The internal standard used was: 4-chloro-3-methylphenol-d2 (QMX (UK)). Water was purified using a Milli-Q purification system from Millipore (Nottingham, UK). Methanol, formic acid (>95 %), HCl (concentrated), 1M NaOH, 1M NH4OH, NH4F, 2-propanol and bisphenol A (BPA) were purchased from Sigma (UK) and Fisher (UK). All solvents used were of LC grade or higher. All the glassware was deactivated using 5 % DMDCS (Sigma, UK) to prevent losses from analyte adsorption. The deactivation procedure consisted of washing the glassware once with 5 % DMDCS followed by two washes with toluene and lastly three washes with methanol.

2.4.2 Sampling and sample collection

Wastewater (untreated, after physical screening) was collected between June and October 2015 from 5 major WWTPs contributing to one river catchment in the South-West UK and covering an area of approximately 2,000 km^2 and the population of ~1.5 million (this constitutes >75% of the overall population in the catchment).

Wastewater was collected on 7 consecutive at each WWTPs as volume proportional 24 h composites with sample collection frequencies of 10 min using ISCO 3700 portable samplers packed with ice (RS Hydro, Worcestershire, UK) (Petrie et al. 2015). All samples were transported to the laboratory on ice for further processing. Grab samples of digested sludge were also collected at one of the sites (Site E) which had anaerobic digestion facilities.

2.4.3 Biomarker stability in wastewater

Biomarker stability in wastewater was performed in PVC bottles in aerobic conditions. Two wastewater aliquots were investigated in duplicate (4° and 17°) to simulate respectively sampler and room temperature. Change in concentration was monitored over 24 h which is the maximum period of time a sample can remain in the sampler awaiting for collection. Samples (100 mL each) were taken at the following interval times (0, 3, 12 and 24h) and prepared for analysis as described below.

2.4.4 Sample preparation and analysis

Liquid samples were filtered using GF/F glass microfibre filter 0.75 µm (Fisher Scientific, UK) and solid phase extraction (SPE) was performed using Oasis HLB (Waters, UK) according to the procedure described elsewhere (Lopardo et al.(Lopardo et al.)). Briefly 100 mL of filtered wastewater were loaded onto HLB cartridges which were preconditioned with 2 mL of MeOH followed by 2 mL of H_2O. After loading the cartridges were dried for 30 minutes and analytes were eluted with 4 mL of MeOH. Extracts were dried using a TurboVap evaporator (Caliper, UK) under a gentle nitrogen stream in a water bath at 40°C then reconstituted in 250 µL 80:20 H_2O:MeOH. The SPE extraction recovery was evaluated at two different concentrations in duplicate using the following equation:

$$Recoveries\ corrected = \frac{(A_{spiked\ before\ SPE} - A_{blank})}{(A_{mobile\ phase})} * 100$$

SPE recoveries were calculated as corrected recoveries (i.e. taking the internal standard concentration into consideration) by the ratio of the concentration of target analytes in wastewater solutions when spiked before SPE (minus the concentration of analyte in the blank wastewater sample), divided by the standard mobile phase concentration.

Digested sludge samples were treated by using microwave assisted extraction (MAE) as described by Petrie et al (Petrie et al. 2015). Briefly samples were frozen, freeze-dried and 0.5 g samples were spiked with 50 ng of 4-chloro-3-methylphenol-D2. Extraction was achieved by heating the samples at 110 ºC using a 800 W MARS 6 microwave (CEM, UK) and 25 mL of 50:50 MeOH:H_2O (pH 2). MAE methanolic extracts were adjusted to <5 % of MeOH using H_2O (pH 2). SPE was performed using Oasis MCX cartridges (Waters, UK) conditioned with 2 mL MeOH followed by 2 mL H_2O (pH 2). Samples were loaded and dried as described previously. Elution was performed using 2 mL 0.6 % HCOOH in MeOH. Once dried extracts were reconstituted in 500 µL 80:20 H_2O:MeOH and filtered using pre-LCMS 0.2 µm PTFE filters (Whatman, Puradisc). Finally samples were analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to (Lopardo et al. (Lopardo et al.)). Briefly, a multi-step gradient was used to separate the analytes at a flow rate of 0.4 mL/min on a BEH C18 column (50 x 2.1 mm, 1.7 µM, Waters UK)

using mobile phase A (1 mM ammonium fluoride in water) and mobile phase B (methanol). The mass spectrometer was equipped with an ESI source and was operated in both positive and negative ionisation mode. The source settings were as follows: capillary voltage was set at 4.5 kV, the end plate offset was set to 500 V, a pressure of 3 Bar was used for the nebulizer gas, the drying gas (nitrogen) flow was 11 L min^{-1} and the drying temperature was set at 220°C. Analysis was run in both full scan mode (MS) and broadband collision induced dissociation (bbCID) mode. Calibrant solution was injected before each run. Quality control samples were run every 10 samples and blanks were run every 3 injections. For details see Lopardo et al. (Lopardo et al.) and Table S 2.3.

2.4.5 Method performance

Linearity was established by triplicate injection of a 13-point calibration curve ranging in concentration from 0.01 to 100 ng mL^{-1}. Instrumental detection limits (IDLs) and instrument quantitation limits (IQLs) were calculated according to the lowest concentration which gave a signal to noise ratio of ≥ 3 and ≥ 10 respectively. Recovery of target chemicals was determined by spiking crude wastewater at a concentration of 4 and 50 ng L^{-1}. BPA and BPA sulphate validation parameters are listed in Table S 2.4 and S 2.5.

2.4.6 Daily mass loads and BPA intake

Daily mass loads of BPA were calculated by multiplying the concentrations (ng L^{-1}) found in a 24 h pooled raw wastewater sample by the daily wastewater flow rate (m^3 day^{-1}). Total BPA concentrations in raw wastewater were calculated taking into account both the amount of BPA absorbed onto solid particulate matter (SPM) and the amount dissolved into liquid fraction. Mass loads (mg day^{-1}) were then normalized to the number of people served by each WWTP (mg/ day/1000 inhabitants), in order to compare results between different WWTPs. Population-wide BPA intakes were calculated using the following equation:

$$BPA_{intake} = \frac{(conc. x\ V) x\ CF}{P}$$

where: conc. is the concentration of BPA sulphate (ng/mL) in influent wastewater, V is the volume of wastewater received by the WWTP per day (m^3/day), P stands for the population served by the WWTP and CF is the correction factor first used by Zuccato

et al. (Zuccato et al. 2008a) to estimate populations drug abuse and later by Rousis et al. (Rousis et al. 2017a) to evaluate population intake of pesticides. CF was calculated taking into account the molecular mass ratio between BPA and BPA sulphate and BPA sulphate excretion ratio. There are published studies that investigated the presence of BPA sulphate in urine. Ye et al. (Ye et al. 2005) found that BPA sulphate represented 21% of BPA metabolites in urine (n=30). Ho et al. (Ho et al. 2017) analysed 140 urine samples finding that BPA sulphate represented 6.25 % of BPA metabolites. Thayer et al. (Thayer et al. 2015) instead observed that only 3% of the total BPA-d6 ingested by 14 volunteers was excreted in urine as BPA sulphate and that the totality of BPA-d6 was excreted within 24h from consumption. CF for BPA sulphate was therefore calculated as 0.161, using 8.41% as BPA sulphate excretion factor (weighed mean of the percentage of BPA sulphated excreted against the number of urine samples in the different studies).

2.5 Results and discussion

2.5.1 BPA sulphate as a biomarker of BPA intake

Bisphenol A is one of the most extensively studied contaminants because of its ubiquity and its suspected endocrine disrupting activity (Joint Fao Oms Expert Committee On Food Additives 2010; Rochester 2013). Because of its toxicity the EFSA (European Food Safety Agency) has temporary lowered the tolerable daily intake (TDI) from 50 μg kg^{-1} day^{-1} to 4 μg kg^{-1} day^{-1} until the outcome of a long-term study will help reducing uncertainties about potential health effects (European Food Safety Authority (EFSA) 2015a). Even though BPA is not intentionally added as ingredient to personal care products or in the food production process, its presence might be due to migration from polycarbonate containers, epoxy resins coating or to the degradation of the BPA-containing material (Hartle et al. 2016; Poustka et al. 2007). Geens et al. (Geens et al. 2012) summarised all the dietary (generally considered to be the main source of BPA) and non-dietary exposure (e.g. dust, thermal paper, dental materials, etc.) (Vandenberg et al. 2007). Christensen et al. (Christensen et al. 2012) concluded that the non-dietary exposure to be one-third of the cohort median exposure. Up to date the extent of the exposure to BPA is generally assessed using two approaches. The first one entails the monitoring of concentrations of contaminants in exposure media with exposure media contact rates (Lu et al. 2018). Interestingly the European Union (Aschberger et al. 2010) and EFSA (European Food Safety Authority (EFSA) 2015b) found the dietary intake to vary significantly with age, indicating major exposure risk for infants (up to 13 μg kg^{-1} day^{-1}) and 1.5 μg kg^{-1} day^{-1} for adults. The second approach relies on human biomonitoring. This approach measures the concentration of BPA and metabolites in biological fluids to back-calculate the overall exposure including both known and unknown sources (Dekant and Völkel 2008).

In this study BPA sulphate was selected as a biomarker of exposure to BPA and BPA loads were monitored alongside. In our previous work (Lopardo et al. (Lopardo et al. 2018)), BPA sulphate was selected as a characteristic metabolic biomarker of human internal exposure to BPA. Research undertaken in this paper confirmed its high stability in wastewater over 24h sampling time at 4°C (Figure S 2.2).

2.5.2 Estimation of public BPA intake via wastewater fingerprinting

BPA and BPA sulphate were identified and quantified (Table S 2.6) in all samples collected from 5 major wastewater treatment plants contributing to a large river catchment in the South-West UK and covering an area of approximately 2,000 km^2 and the population of ~1.5 million (this constitutes >75% of the overall population in the catchment). BPA sulphate was found at higher concentrations than parent BPA, possibly due to higher hydrophobicity and sorption potential to SPM in the case of BPA(Petrie et al. 2019). Its concentrations ranged from 0.7 to 6 µg L^{-1} for WWTPs A, C and D and from 3 to 120 µg L^{-1} for WWTPs B and E. BPA was found at much lower concentrations than its metabolite with an exception of WWTP E. Concentrations of BPA ranged from 0.5 to 0.9 µg L^{-1} in WWTPs A and C, while in WWTPs B, D and E they ranged from 1.3 to 51 µg L^{-1} with the highest concentrations recorded in WWTP E being circa 100 µg L^{-1}. Recorded SPM concentrations ranged from 0.03 to 0.27 µg L^{-1} in WWTPs A, C and D while in WWTPs B, and E they ranged from 0.1 to 7.7 µg L^{-1} with the highest concentrations recorded in WWTP E being circa 15 µg L^{-1}.

Analysis of the liquid fraction of activated sludge and digested sludge revealed that only a minor fraction of BPA sulphate adsorbs onto solid matter (< 7%). That was expected given the greater hydrophilicity of BPA sulphate compared to BPA.

Due to sampling being conducted in WWTPs located in 5 different geographical locations within the same river catchment, considerable spatial variability in daily BPA and BPA sulphate loads could be observed and because sampling at each WWTP was operated over 7 consecutive days (Wednesday to Tuesday) it was possible to gather information about both spatial and temporal variability for a full week. BPA loads ranged from 180 to 600 mg day^{-1} per 1000 inhabitants at WWTPs A, C and D. At WWTP E, BPA loads were on average two orders of magnitude greater but on weekends lower loads were registered. WWTP B showed the greatest variability with BPA loads ranging from 500 to 7000 mg day^{-1} per 1000 inhabitants (Figure 3). These loads correspond to concentrations of BPA in wastewater up to 100 µg L^{-1} which is considerably more than what is generally observed since BPA can be usually found in urban wastewater at concentrations around 1 µg L^{-1} (Gatidou et al. 2007; Lopardo et al.; Petrie et al. 2015). The reason why such high concentrations of BPA were observed at WWTPs B and E might be industrial wastewater contribution as suggested by Petrie

et al. (Petrie et al. 2018). Higher BPA loads were in fact observed downstream from a paper producing plant from Fuerhacker in 2003 (Fuerhacker 2003). Interestingly, the high load of BPA at WWTP E did not correspond with high loads of BPA sulphate (Figure 2.2). These loads correspond to concentrations of BPA in wastewater up to 120 μg L^{-1} which is considerably more than what is generally observed since BPA can be usually found in urban wastewater at concentrations around 1000 ng L^{-1} (Gatidou et al. 2007; Lopardo et al.; Petrie et al. 2015). The reason why such high concentrations were observed at WWTPs B and D might be industrial wastewater contribution. Higher BPA loads were in fact observed downstream a paper producing plant from Fuerhacker in 2003 (Fuerhacker 2003). In fact, two high loads of BPA did not correspond high loads of BPA sulphate (see WWTP D, Figure 2.4). Average BPA sulphate loads ranged instead at WWTPs A, C and E from 469 to 682 mg day^{-1} per 1000 inhabitants (Figure 2.2) whereas average loads at WWTPs B and D were an order of magnitude greater (from 4633 to 5347 mg/day per 1000 inhabitants, Figure 2.2). Temporal variability (calculated as RSD %) below 40 % was observed at WWTPs A and D (Figure 2.2). This showed BPA sulphate loads here were relatively consistent supporting the hypothesis that population exposure pattern is also relatively consistent throughout the week. On the other hand, BPA sulphate loads variability was higher at other WWTPs (ranging from 50 to 131 %), indicating generally lower exposure during weekends compared to weekdays (Figure 2.2). In particular at WWTPs D and E loads were registered to be 6 to 7 times lower on Saturdays and Sundays compared to Mondays to Thursdays. WWTP B showed the greatest variability due to loads being more than an order of magnitude greater on two weekdays.

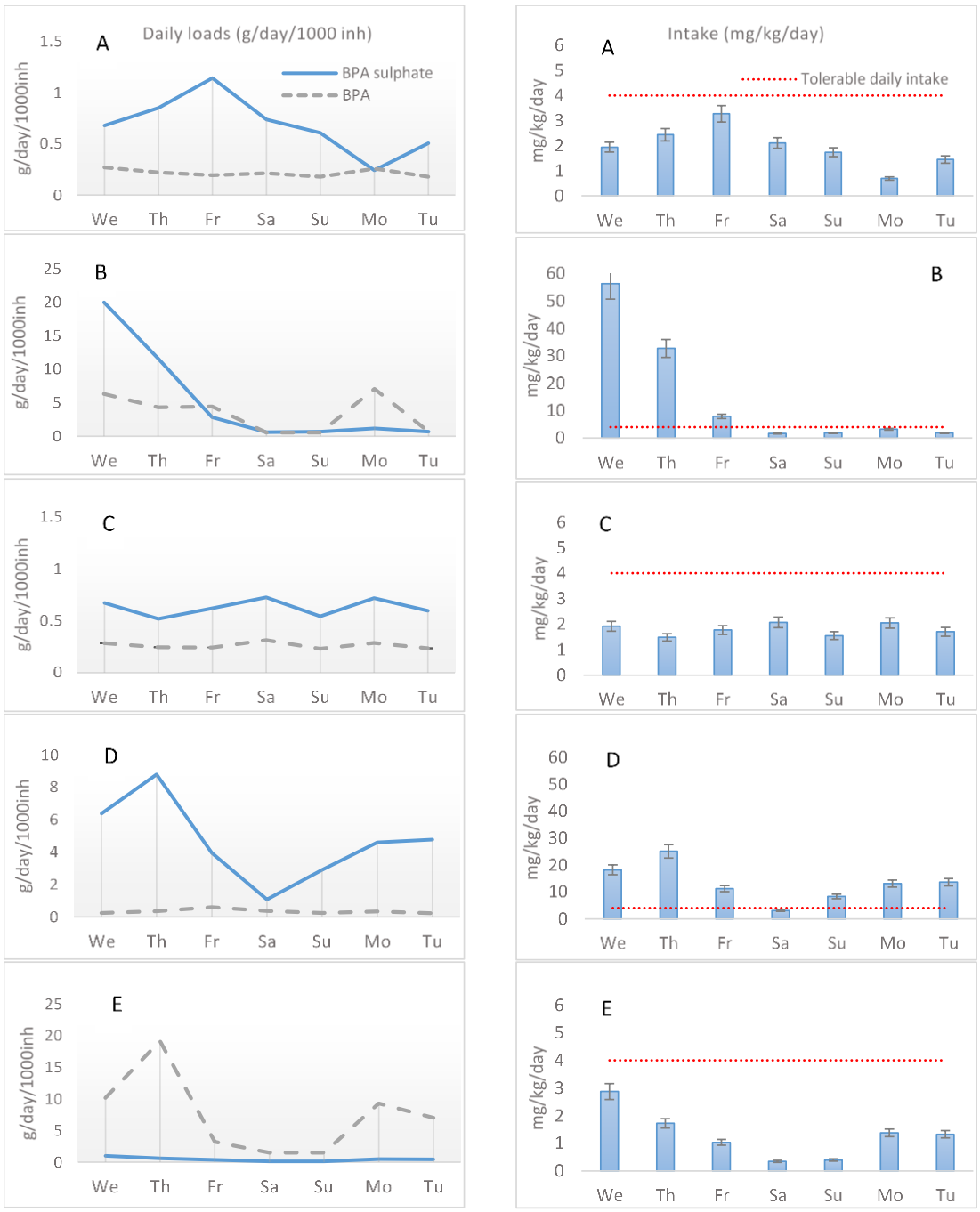


Figure 2.2 Temporal variability of BPA and BPA sulphate influent wastewater loads (left) and estimated BPA daily intake (right) during 7 consecutive days in the 5 WWTPs investigated namely A, B, C, D and E serving a population of respectively 37,000; 67,870; 105,847; 17,638; 909,617.

2.5.3 Estimation of public BPA intake via wastewater fingerprinting

Community-wide BPA intake (internal exposure) was estimated using the WBE (wastewater-based epidemiology) approach as described in section 2.5. Calculated loads of BPA sulphate at WWTPs A, C and E corresponded to an estimated BPA intake (Figure 2.2) which was consistently below TDI level set by EFSA (at 4 μg kg^{-1} day^{-1}) suggesting overall low exposure. On the other hand, WWTPs B and D were characterised by higher loads corresponding to higher intakes that were above the TDI threshold for several days during the sampling period. WWTP B results indicate that accidental release of BPA linked with elevated exposure occurred on or just before Wednesday. On the other hand, WWTP D shows overall high exposure. Further work will need to be undertaken in this particular location to verify possible BPA exposure patterns, as well as to fully understand sources of BPA sulphate that might be contributing to the biomarker concentration levels in the studied catchment.

It is also interesting to note that characteristic temporal variations of BPA intake are observed in all studied WWTPs with the lowest intake occurring during weekends and the highest during weekdays. This is an important observation indicating that public exposure to BPA is much higher during working days. There are several possible reasons for this including healthier diet during weekends vs higher exposure of workers in industrial settings during weekdays.

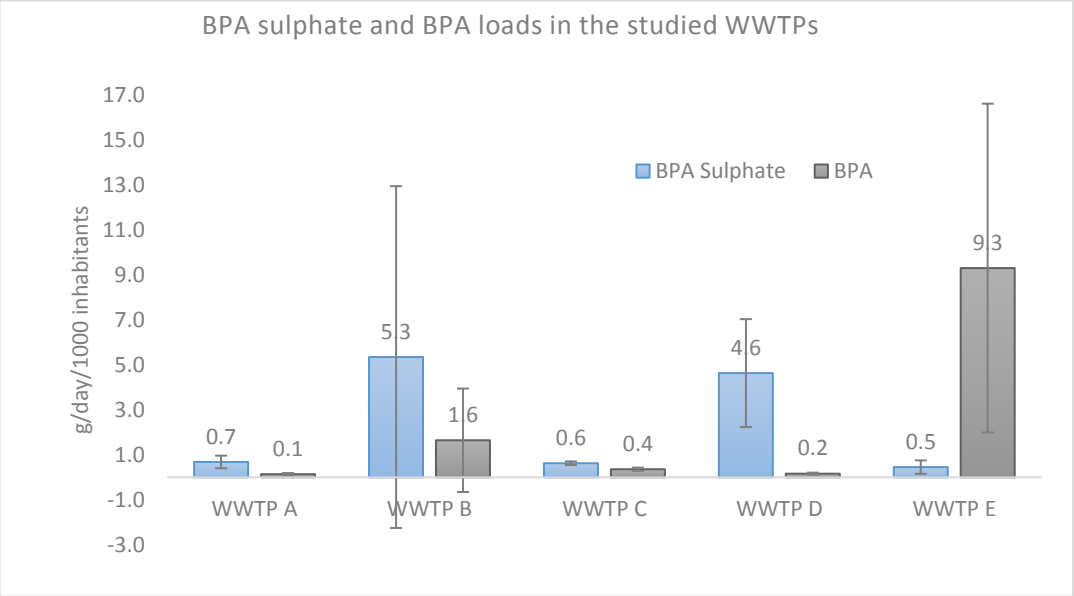


Figure 2.3 Comparison between weekly average BPA and BPA sulphate loads in the 5 WWTPs investigated

2.5.4 Cumulative BPA loads versus daily BPA intake

Comparison of population normalised daily BPA and BPA sulphate loads revealed that the presence of BPA in wastewater allowing for estimation of community wide external BPA exposure is not indicative of its actual intake. As can be seen in Figure 2.3, BPA loads in WWTP B and E are higher due to possible industrial contribution. On the other hand, WWTPs B and D have the highest loads of BPA sulphate (indication of exposure).

2.6 Conclusions

This study provided comprehensive spatiotemporal community-wide exposure to BPA (including BPA intake) using wastewater fingerprinting applied to a large geographical area of 2,000 km^2 and a population of ~1.5 million accounting for >75% of the overall population in the studied catchment. Community-wide BPA intake was found to be consistently below TDI level set by EFSA (at 4 $\mu g\ kg^{-1}\ day^{-1}$) suggesting overall low exposure in most locations. However, at two WWTPs, higher BPA sulphate loads corresponding to higher intakes that were above the TDI threshold were observed. Characteristic temporal variations of BPA intake were observed in most studied WWTPs with the lowest intake occurring during weekends and the highest during weekdays. This is an important observation indicating that public exposure to BPA is much higher during working days. There are several possible reasons for this including healthier diet during weekends vs higher exposure of workers in industrial settings during weekdays. Further work will need to be undertaken to fully understand sources of BPA and BPA sulphate that might be contributing to the biomarker concentration levels in the studied catchment.

2.7 Acknowledgments

The support of the Leverhulme Trust (Project No RPG-2013-297), Wessex Water and the University of Bath's EPSRC Impact Acceleration Account (Project number: EP/K503897/1 and ZR-Z0248) is greatly appreciated. We also acknowledge the Environment Agency for providing river flow data (Contains Environment Agency information © Environment Agency and database right). All data supporting this study are provided as supporting information accompanying this paper.

2.8 Supplementary material

2.8.1 List of figures

2.8.2 List of tables

Table S 2.1 Endocrine disruptors in household products and suggested biomarkers for WBE

	Endocrine disruptor	Household products[1]	Excretion	Proposed biomarkers of exposure	Notes (e.g. regulation)[2]
Antimicrobial (Hiles et al. 1978; Moss et al. 2000; Pycke et al. 2015; Sandborgh-Englund et al. 2006; Wang et al. 2009; Wu et al. 2010; Ye et al. 2011)	Triclocarban	bar soap antiperspirant/ deodorant liquid hand soap	Urine and feaces	2'-OH-Triclocarban 3'-OH-Triclocarban Sulphated Glucuronidated	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): triclosan; 2,4,4'-trichloro-2'-hydroxy-diphenyl-ether; 5-chloro-2-(2,4-dichlorophenoxy)phenol Index No(s). 604-070-00-9 Classification: Hazard Class and Category Code(s): Skin Irrit. 2 , Eye Irrit. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H315, H319, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 25 of Annex V is amended.

[1] Data collected from http://www.ewg.org and http://www.householdproducts.nlm.nih.gov/index.htm

[2] Data collected from https://scifinder.cas.org/

Triclosan	liquid hand soap antiperspirant/ deodorant toothpaste body wash/ cleanser acne treatment moisturizer shaving cream dishwashing liquid	Urine and feaces	Sulphate conjugated Glucuronidated	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): triclosan; 2,4,4'-trichloro-2'-hydroxy-diphenyl-ether; 5-chloro-2-(2,4-dichlorophenoxy)phenol Index No(s). 604-070-00-9 Classification: Hazard Class and Category Code(s): Skin Irrit. 2 , Eye Irrit. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H315, H319, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 25 of Annex V is amended.
4-Cl-3,5-dimethylphenol (chloroxylenol)	Hand cleaner	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): 4-chloro-3,5-dimethylphenol Index No(s). 604-038-00-4 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Skin Irrit. 2 , Skin Sens. 1, Eye Irrit. 2 Hazard Statement Code(s): H302, H315, H317, H319. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex V, List of Preservatives Allowed in Cosmetic Products.

Flame retardant (Retardant et al. 2014; Sandholm et al. 2003; Schauer et al. 2006a)	Tetrabromo bisphenol A (TBBPA)		Byproduct/Intermediate/Reactant, Flame retardant, Household product ingredient, Plastic/Rubber	< 0.1% Urine < 80 % feces	Glucuronidated Di-glucuronidated Sulphated	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): tetrabromobisphenol-A; 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol Index No(s). 604-074-00-0 Classification: Hazard Class and Category Code(s): Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H400, H410. **European Community Legislation** Official Journal of the European Union, No C 152 #51:2 (18 Jun 2008). In accordance with Council Regulation (EEC) No. 793-93, the risk evaluation has been completed and reduction measures for workers and the environment for this substance have been recommended.
	Bis-(pentabromophenyl)ether (BDE209)		Flame retardant, Household product ingredient, Plastic/Rubber	> 90 % feces	Hydroxylated Guiacol	**European Community Legislation** Official Journal of the European Union, No L 35 #60:6 (10 Feb 2017). Effective date: 02 MAR 2017 Commission regulation(EU) 2017/227 of 09 February 2017 mending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(pentabromophenyl)ether. Annex XVII to Regulation (EC) No 1907/2006 is amended in accordance with the Annex to this Regulation.

	Hexabromocyclododecane (HBCD)		Flame retardant, Household product ingredient, Plastic/Rubber	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): 1,2,5,6,9,10-hexabromocyclododecane Index No(s). 602-109-00-4 Classification: Hazard Class and Category Code(s): Repr. 2, Effect on or via lactation Hazard Statement Code(s): H361, H362.
Fragrances (Bitsch et al. 2002; Fernandes et al. 2013; Yin et al. 2012)	Musk xylene		Used as fragrance ingredients in washing and cleaning agents products for personal care and in other consumer products	Not known	o- Amino musk xylol p- Amino musk xylol	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): musk xylene; 5-tert-butyl-2,4,6-trinitro-m-xylene Index No(s). 609-068-00-1 Classification: Hazard Class and Category Code(s): Expl. Div. 1.1, Carc. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H201, H351, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex III, List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down.

Musk ketone	Used as fragrance ingredients in washing and cleaning agents products for personal care and in other consumer products	Not known	2- amino musk ketone	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): musk ketone; 3,5-dinitro-2,6-dimethyl-4-tert-butylacetophenone; 4'-tert-butyl-2',6'-dimethyl-3',5'-dinitroacetophenone Index No(s). 609-069-00-7 Classification: Hazard Class and Category Code(s): Carc. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H351, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex III, List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down.
Galaxolide	anti-aging facial moisturizer/ treatment exfoliant/ scrub	Not known	Hydroxilated Galaxolide	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran; galaxolide; (HHCB) Index No(s). 603-212-00-7 Classification: Hazard Class and Category Code(s): Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H400, H410. **European Community Legislation** Official Journal of the European Union, No L 97 (05 Apr 2006). This substance is listed in Section 1, Annex I (Cosmetic Ingredients other than Perfume and Aromatic Raw Material) of Commission Directive 2006/257/EC, an amendment of Section 5a of Commission Directive 76/768/EEC
		Breast milk	Lactone	

						which establishes the inventory and common nomenclature of ingredients used in cosmetic products. INCI Name: HEXAHYDROHEXAMETHYL CYCLOPENTABENZOPYRAN Function: antifoaming.
	Tonalide		Household product ingredient, Personal care product/Cosmetic ingredient, Pesticide ingredient	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 114 #56:1 (25 Apr 2013). Commission Implementing Regulation (EU) No 344/2013 of 4 April 2013 amending Annexes II, III, V, and VI to Commission Implementing Regulation (EC) No 1223/2009. This substance is listed on Annex III, List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down.
Pesticides(Abel et al. 2004; Barr et al. 2007; Buchholz et al. 1999; Catenaccia et al. 1993; Molina-Molina et al. 2006; Will 1995; Wong et al. 1995)		Atrazine	Food Drinking water	Urine	Dealkylated Atrazine Bi-dealkylated Atrazine Atrazine Mercapturate Atrazine Hydroxylated	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): atrazine; 2-chloro-4-ethylamine-6-isopropylamine-1,3,5-triazine Index No(s). 613-068-00-7 Classification: Hazard Class and Category Code(s): Skin Sens. 1, STOT Rep. Exp. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H317, H373, H400, H410. **European Community Legislation** Official Journal of the European Union L 204 #51:15 (31 Jul 2008). This substance is added to Annex I, Part 2 (List of chemicals subject to export notification procedure) of Regulation (EC) No. 304/2003. This chemical qualifies for Prior Informed Consent (PIC) notification.

	Vinclozolin	Food	Urine	2-[(3,5-dichlorophenyl)-carbamoyl-oxy]-2-methyl- 3-butenoic acid (3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide)	**Hazard, Toxicology, and Use Information** Agricultural Chemical Fungicide, bactericide, wood preservative Mutation data Reproductive Effect (RTECS) Health Hazards Fed. Regist. 78 #115:35928 (14 Jun 2013). This substance is included in the final second list of chemicals subject to Tier 1 screening under the Endocrine Disruptor Screening Program (EDSP). High Production Volume Chemicals Internet: oecd.org, October 2009. This substance is listed on the 2007 OECD List of High Production Volume Chemicals. This substance was produced at a level greater than 1,000 tons/year in at least one member country of the European Union. It is a high production volume (HPV) chemical.

	Bifenthrin	Food Fertilizers	Not known	4-hydroxy bifenthrin	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): bifenthrin; (2-methylbiphenyl-3-yl)methyl rel-(1R,3R)-3-[(1Z)-2-chloro-3,3,3-trifluoroprop-1-en-1-yl]-2,2-dimethylcyclopropane carboxylate Index No(s). 607-699-00-7 Classification: Hazard Class and Category Code(s): Acute Tox. 2, Skin Sens. 1B, Acute Tox. 3, Carc. 2, STOT Rep. Exp. 1, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H300, H317, H331, H351, H372, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 31 #60:21 (04 Feb 2017). Effective date: 24 FEB 2017 Commission Implementing Regulation (EU) No 2017/195 of 03 February 2017, amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of this active substance, Bifenthrin. Part B of the Annex to Implementing (EU) No 540/2011 is amended in accordance with the Annex to this Regulation. The expiration date of the approval period has been changed to 31 July 2021. The measures provided for in this regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed.

Plasticizers(González-Mariño et al. 2017; Silva et al. 2006, 2007)	Di-n-butyl- phthalate (DBP)	Nail Products Plastic food containers Children's toys	Urine	Mono(3-hydroxybutyl) phthalate	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): dibutyl phthalate; DBP Index No(s). 607-318-00-4 Classification: Hazard Class and Category Code(s): Repr. 1B, Aquatic Acute 1 Hazard Statement Code(s): H360Df, H400. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex II, List of Substances Prohibited in Cosmetic Products.
	Bisphenol A diglycidyl ether (BADGE)		~45% of total BADGE excreted in urine	BADGE • 2H2O	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): bis-[4-(2,3-epoxipropoxi)phenyl]propane Index No(s). 603-073-00-2 Classification: Hazard Class and Category Code(s): Skin Irrit. 2 , Skin Sens. 1, Eye Irrit. 2 Hazard Statement Code(s): H315, H317, H319.
	Bis(2-ethylhexyl) phthalate (DEHP)	Plastic food containers Children's toys	~29.7 % of total DEHP excreted in urine ~31.8 % of total DEHP excreted in urine ~15.3 % of total DEHP excreted in urine	mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) mono (2-ethyl-5-carboxypentyl) phthalate (MECPP) mono (2-ethyl-5-oxohexyl) phthalate (MEOHP)	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): bis(2-ethylhexyl) phthalate; di-(2-ethylhexyl) phthalate; DEHP Index No(s). 607-317-00-9 Classification: Hazard Class and Category Code(s): Repr. 1B Hazard Statement Code(s): H360FD.

Preservatives(Janjua et al. 2008; Ye et al. 2006)	Methylparaben	Eye make-up; Blush/foundation; Hair products; Facial moisturizer/ treatment; Preservative in food and food dyes	Urine	Sulphated and glucuronidated	**European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 12 of Annex V is amended.
	Ethylparaben	Eye make-up; Blush/foundation; Hair products; Facial moisturizer/ treatment; Preservative in food and food dyes	Urine	Sulphated and glucuronidated	**European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 12 of Annex V is amended.
	Propylparaben	Eye make-up; Blush/foundation; Hair products; Facial moisturizer/ treatment; Preservative in food and food dyes	Urine	Sulphated and glucuronidated	**European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 12 of Annex V is amended.
	Butylparaben	Eye make-up; Blush/foundation; Hair products; Facial moisturizer/ treatment; Preservative in food and food dyes	Urine	Sulphated and glucuronidated	**European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. Substance 12 of Annex V is amended.

		Benzylparaben	Medical/Veterinary/Research, Personal care product/Cosmetic ingredient	Urine	Sulphated and glucuronidated	**European Community Legislation** Official Journal of the European Union, No L 107 #57:5 (10 Apr 2014). Commission Implementing Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Commission Implementing Regulation (EC) No 1223/2009. This substance is listed on Annex II, List of Substances Prohibited in Cosmetic Products.
Surfactant(Mu et al. 1998; Ye et al. 2007)	4-n-nonylphenol		Byproduct/Intermediate/Reactant, Industrial additive, Medical/Veterinary/ Research, Metabolite/Degradate, Pesticide ingredient, Plastic/Rubber	Urine	4-n-nonylphenol hydroxylated 4-n-nonylphenol sulphate 4-n-nonylphenol glucuronidates	**European Community Legislation** Official Journal of the European Union L 204 #51:15 (31 Jul 2008). This substance is added to Annex I, Part 2 (List of chemicals subject to export notification procedure) of Regulation (EC) No. 304/2003. This chemical qualifies for Prior Informed Consent (PIC) notification.
	4-n-octylphenol		Byproduct/Intermediate/Reactant, Industrial additive, Plastic/Rubber	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 33 (04 Feb 2006). Notice of Regulation (EC) No. 166/2006 of the European Parliament and of the Council of the European Union of 18 January 2006 concerning the establishment of a European Pollutant Release and Transfer Register (European PRTR) and amending Council Directives 91/689/EEC and 96/61/EC. This Regulation establishes the European PRTR to implement the UNECE Protocol on Pollutant Release and Transfer Registers. The effective date is 24 February 2006. This substance is listed in Annex II.

Perfluorooctanoic acid (PFOA)		Byproduct/Intermediate/Reactant, Household product ingredient, Industrial additive, Metabolite/Degradate, Personal care product/Cosmetic ingredient	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): perfluorooctanoic acid Index No(s). 607-704-00-2 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Eye Damage 1, Acute Tox. 4, Carc. 2, Repr. 1B, Effect on or via lactation, STOT Rep. Exp. 1 Hazard Statement Code(s): H302, H318, H332, H351, H360D, H362, H372. **European Community Legislation** Official Journal of the European Union, No L 150 #60:14 (14 Jun 2017). Effective date: 04 JUL 2017 Commission Implementing Regulation (EU) No. 2017/1000 of 13 June 2017 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards perfluorooctanoic acid (PFOA), its salts and PFOA-related substances. Annex XVII to Regulation (EC) No 1907/2006 is amended in accordance with the Annex to this Regulation. The measures provided for in this Regulation are in accordance with the opinion of the Committee established under Article 133 of Regulation (EC) No 1907/2006.

	nonylphenol diethoxylate		Household product ingredient, Pesticide ingredient	Not known	Not known	**German Water Hazard Class Substance List** German Water Hazard Class Substances List, 27 Nov 2006. This substance has been assigned a Water Hazard Classification under the June 1999 Administrative Regulation on Substances Hazardous to Waters (Verwaltungsvorschrift wassergefahrdende Stoffe) in Germany. Listed Name(s): Nonylphenolethoxylate. Kenn-Number: 671 State of Classification: VwVwS. German Water Hazard Classification (WGK) Number: 2.
Antiseptic (Lopardo et al. 2018)	2-naphthol		Antimicrobial, Byproduct/Intermediate/Reactant, Industrial additive, Medical/Veterinary/Research, Metabolite/Degradate, Plastic/Rubber	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): 2-naphthol Index No(s). 604-007-00-5 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Acute Tox. 4, Aquatic Acute 1 Hazard Statement Code(s): H302, H332, H400. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex II, List of Substances Prohibited in Cosmetic Products.

	Chlorothymol	Antimicrobial, Household product ingredient, Medical/Veterinary /Research, Personal care product/Cosmetic ingredient	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 97 (05 Apr 2006). This substance is listed in Section 1, Annex I (Cosmetic Ingredients other than Perfume and Aromatic Raw Material) of Commission Directive 2006/257/EC, an amendment of Section 5a of Commission Directive 76/768/EEC which establishes the inventory and common nomenclature of ingredients used in cosmetic products. INCI Name: CHLOROTHYMOL Function: antimicrobial/denaturant/deodorant/oral care/antiplaque.
	4-benzylphenol	Medical/Veterinary /Research, Plastic/Rubber	Not known	Hydroxylated Sulphated Glucuronated Hydroxylated and conjugated	Non regulated
Bactericide(Lopardo et al. 2017)	2,4,6-trichlorophenol	Antimicrobial, Byproduct/Intermediate/Reactant, Household product ingredient, Industrial additive, Pesticide ingredient	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): 2,4,6-trichlorophenol Index No(s). 604-018-00-5 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Skin Irrit. 2, Eye Irrit. 2, Carc. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H302, H315, H319, H351, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex II, List of Substances Prohibited in Cosmetic Products.

	4-chloro-3-methylphenol	Moisturizer anti-fungal treatment shaving cream	Urine	Hydroxylated Sulphated Glucuronidated	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2015. This is the harmonized C&L classification. Listed Name(s): chlorocresol; 4-chloro-m-cresol; 4-chloro-3-methylphenol Index No(s). 604-014-00-3 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Acute Tox. 4, Skin Sens. 1, Eye Damage 1, Aquatic Acute 1 Hazard Statement Code(s): H302, H312, H317, H318, H400. **European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex V, List of Preservatives Allowed in Cosmetic Products.
Funcicide	2,4,5-trichlorophenol	Antimicrobial, Industrial additive, Medical/Veterinary /Research, Pesticide ingredient	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): 2,4,5-trichlorophenol Index No(s). 604-017-00-X Classification: Hazard Class and Category Code(s): Acute Tox. 4, Skin Irrit. 2, Eye Irrit. 2, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H302, H315, H319, H400, H410.

	Prochloraz	Pesticide ingredient	Not known	Not known	**EU Classification, Labeling and Packaging** Regulation (EC) No. 1272/2008 Internet: echa.europa.eu, 2016. This is the harmonized C&L classification. Listed Name(s): prochloraz; N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]-1H-imidazole-1-carboxamide Index No(s). 613-128-00-2 Classification: Hazard Class and Category Code(s): Acute Tox. 4, Aquatic Acute 1, Aquatic Chronic 1 Hazard Statement Code(s): H302, H400, H410. **European Community Legislation** Official Journal of the European Union, No L 325 (11 Dec 2007). Publication of Council Directive 98/8/EC Article 16(2) concerning the placing of biocidal products on the market. This substance is included in Annex I; classified as an existing active substance in biocidal products in accordance with the requirements of Article 3(1) or 5(2) of Regulation (EC) No 1896/2000.
Cosmetic	2-Ethylhexyl 4-(dimethylamino)benzoate (Padimate O)	Lip cosmetics Hair products sunscreen	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 8 %

	homosalate		sunscreen tanning oil Lip cosmetics	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 10 %
UV-filters (Dewalque et al. 2014; Gonzalez et al. 2008; León et al. 2010; Lopardo et al. 2018; Morrison et al. 2017; Schauer et al. 2006b; Ye et al. 2005)	3-benzylidene camphor		Household product ingredient, Medical/Veterinary /Research, Personal care product/Cosmetic ingredient	Not known	3-benzylidene camphor hydroxylated	**European Community Legislation** This substance is included in Annex II, List of Substances Prohibited in Cosmetic Products
	3-(4-Methylbenzylidene) camphor (4-MBC)		Personal care product/Cosmetic ingredient	Urine	3-(4-carboxybenzylidene)camphor glucuronide 3-(4-carboxybenzylidene)-6-hydroxycamphor	**European Community Legislation** This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 4 %
	Benzophenone-1		Food additive, Household product ingredient, Metabolite/Degradate, Personal care product/Cosmetic ingredient, Pesticide ingredient, Plastic/Rubber	Not known	Hydroxylated Sulphated Glucuronated Hydroxylated and conjugated	**European Community Legislation** Official Journal of the European Union, No L 97 (05 Apr 2006). This substance is listed in Section 1, Annex I (Cosmetic Ingredients other than Perfume and Aromatic Raw Material) of Commission Directive 2006/257/EC, an amendment of Section 5a of Commission Directive 76/768/EEC which establishes the inventory and common nomenclature of ingredients used in cosmetic products. INCI Name: BENZOPHENONE-1 Function: uv absorber.
	Benzophenone-2		Food additive, Household product ingredient, Personal care product/Cosmetic	Not known	BP-2 Sulphated	**European Community Legislation** Official Journal of the European Union, No L 97 (05 Apr 2006). This substance is listed in Section 1, Annex I

		ingredient, Pesticide ingredient, Plastic/Rubber			(Cosmetic Ingredients other than Perfume and Aromatic Raw Material) of Commission Directive 2006/257/EC, an amendment of Section 5a of Commission Directive 76/768/EEC which establishes the inventory and common nomenclature of ingredients used in cosmetic products. INCI Name: BENZOPHENONE-2 Function: uv absorber.
	Benzophenone-3	Personal care products including sunscreen, cosmetics, lotions, fragrances, shampoos, body washes, soap, and insect repellents	Urine	2,4-Dihydroxybenzophenone 2,3,4-Trihydroxybenzophenone Benzophenone-3 sulphate Benzophenone-3 glucuronated	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 10 % Wording of Warning Statement: Contains Benzophenone-3
	Benzophenone-4	Household product ingredient, Personal care product/Cosmetic ingredient	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 5 % (as acid)
	4,4'-Dihydroxybenzophenone	Industrial additive, Personal care product/Cosmetic ingredient, Plastic/Rubber	Not known	Hydroxylated Sulphated Glucuronated Hydroxylated and conjugated	Not regulated

Ethylhexyl methoxycinnamate (EHMC, OMC)		Personal care product/Cosmetic ingredient	Not known	Not known	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 10 %
Octocrylene (OC)		Personal care product/Cosmetic ingredient	Not known	Hydrolytic product	**European Community Legislation** Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 10 % (as acid)
Phenylbenzimidazolesulfonic acid (PBSA; Ensulizole)		Personal care product/Cosmetic ingredient	Not known	Not known	Official Journal of the European Union, No L 342 #52:59 (22 Dec 2009). Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council on 30 November 2009 concerning cosmetic products. This substance is included in Annex VI, List of UV Filters Allowed in Cosmetic Products. Maximum Concentration in Ready to Use Preparation: 8 % (as acid)

Table S 2.2 General information about BPA sulphate

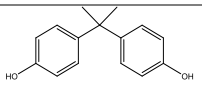
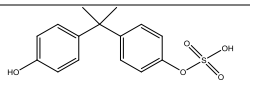
Chemical	CAS number	Molecular Weight	Formula	Structure
Bisphenol A	80-05-7	228.3	$C_{15}H_{16}O_2$	
Bisphenol A sulphate	847696-37-1	308.1	$C_{15}H_{16}O_5S$	

Table S 2.3 UHPLC-QTOF parameters used in the determination of BPA and BPA sulphate

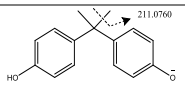
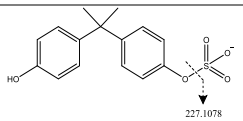
Chemical	Rt (min)	m/z $[M-H]^-$	Mass error (ppm)	SRM transition	Fragment Structure
Bisphenol A	7.7	227.1078	< 10	227.1078 > 211.0760	
Bisphenol A sulphate	6.8	307.0646	<10	307.0646 > 227.1078	

Table S 2.4 UHPLC-QTOF instrument performance parameters

Analyte	IS	Linearity Range [µg L^{-1}]	R^2	Accuracy[1] [%]	Precision[1] [%]	IDL [µg L^{-1}]	IQL [µg L^{-1}]
BPA	4-chloro-3-methylphenol-d2	0.28 – 28.5	0.9972	114.8	2.5	0.08	0.28
Bisphenol A Sulphate	4-chloro-3-methylphenol-d2	1.39 - 103.4	0.9972	98.3	2.1	0.41	1.39

**concentration levels: 0.1, 5 and 100 ng/mL used for precision and accuracy*

Table S 2.5 SPE-UHPLC-QTOF method performance parameters

Analyte	Wastewater SPE recovery [%]*	MDL [ng L^{-1}]	MQL [ng L^{-1}]
BPA	100.4±2.6	0.003	0.007
BPA sulphate	63.7±6.3	0.016	0.055

** based on duplicate extractions at two concentration levels*

Table S 2.6 BPA and BPA sulphate concentrations in wastewater

Analyte	Concentration [ng L^{-1}]				
	WWTPA	WWTPB	WWTPC	WWTP D	WWTP E
BPA	1512.8±291	951±215	666.9±178	11354.7±10526	53890.3±39180
BPA sulphate	3061.8±1170	32396.9±42505	2663.9±422	27947.6±13756	2788.2±1778

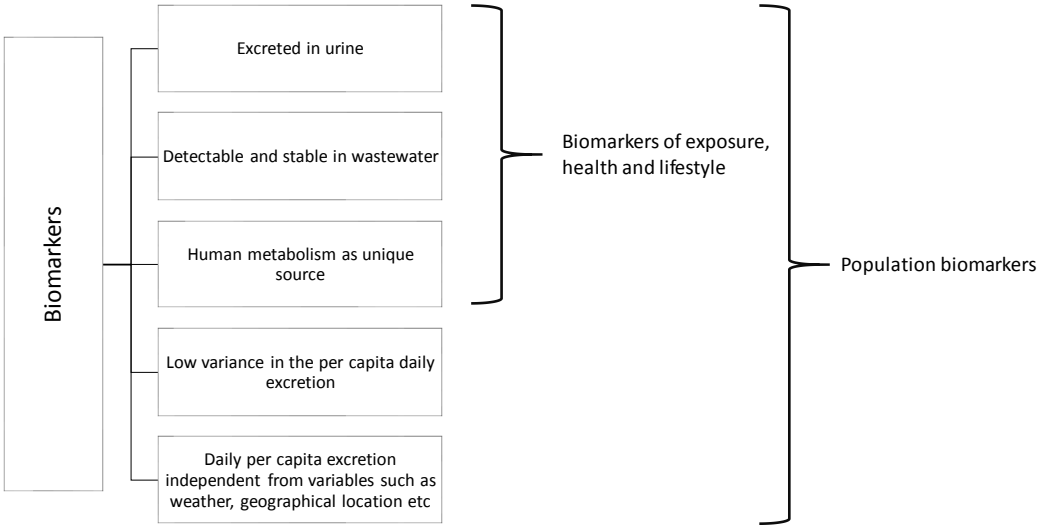


Figure S 2.1 Main requirements of biomarkers modified from [31]

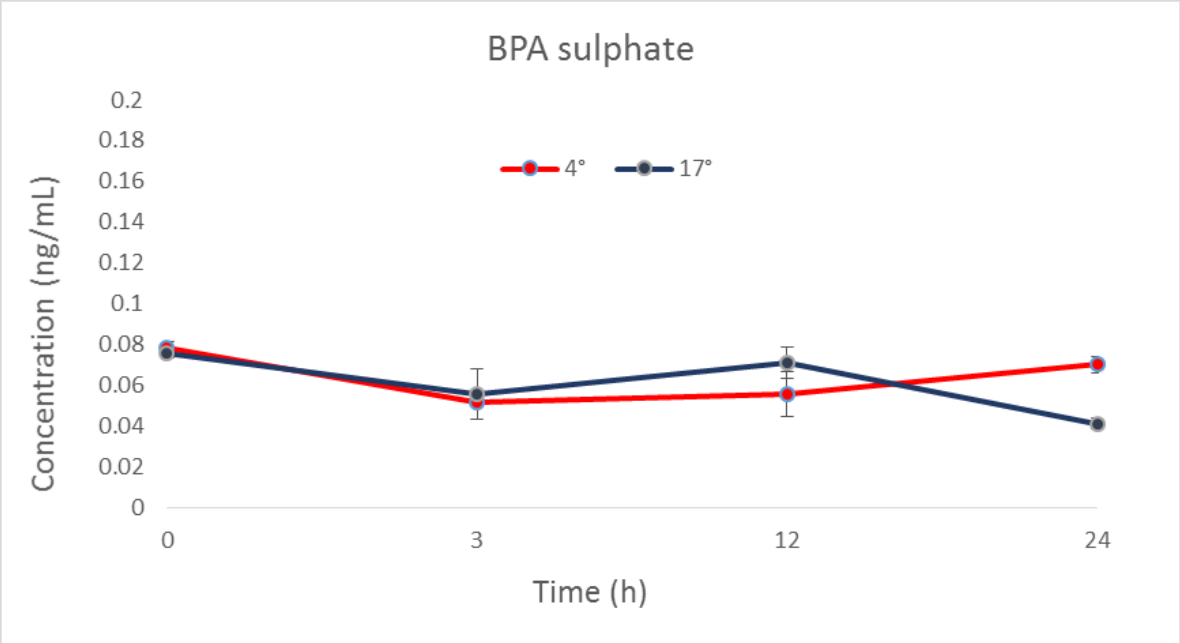


Figure S 2.2 BPA sulphate concentration change over a 24h stability study

2.9 References

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3 A new analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting

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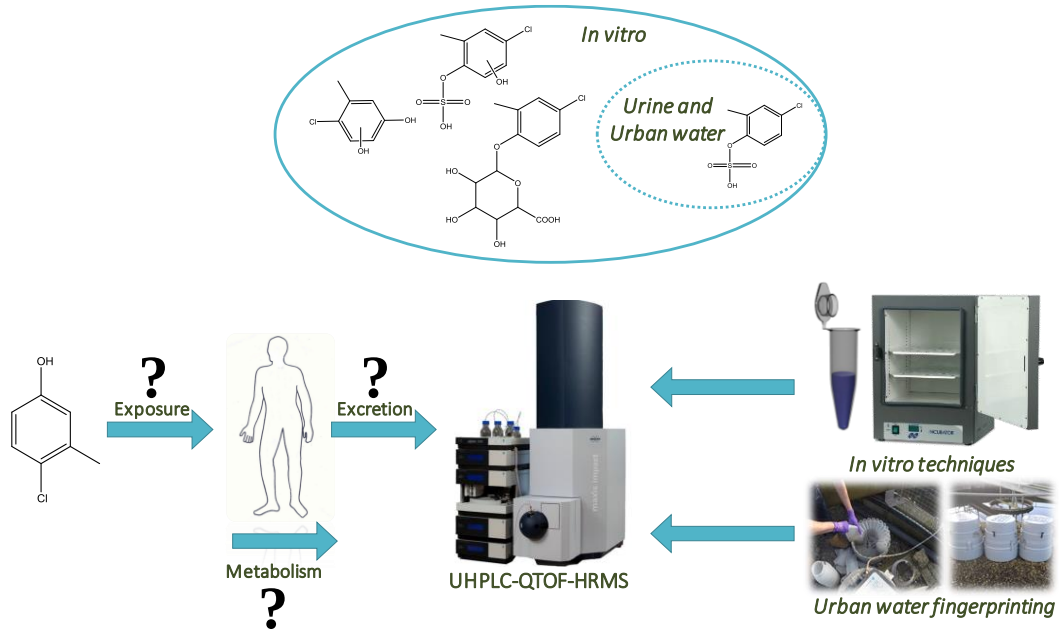
3.1 Graphical abstract



Keywords: wastewater, epidemiology, exposure, antimicrobial agents, endocrine disruptors, environment

3.2 Abstract

Molecular epidemiology approaches in human biomonitoring are powerful tools that allow for verification of public exposure to chemical substances. Unfortunately, due to logistical difficulties and high cost, they tend to evaluate small study groups and as a result might not provide comprehensive large-scale community-wide exposure data. Urban water fingerprinting provides a timely alternative to traditional approaches. It can revolutionise the human exposure studies as urban water represents collective community-wide exposure. Knowledge of characteristic biomarkers of exposure to specific chemicals is key to the successful application of water fingerprinting. This study aims to introduce a novel conceptual analytical framework for identification of biomarkers of public exposure to chemicals via combined human metabolism and urban water fingerprinting assay. This framework consists of: Step 1 - In vitro HLM/S9 assay; Step 2 – In vivo pooled urine assay; Step 3 - In vivo wastewater fingerprinting assay; Step 4 - Analysis with HR-MSMS; Step 5 - Data processing and Step 6 - Selection of biomarkers. The framework was applied and validated for PCMC (4-chloro-m-cresol), household derived antimicrobial agent with no known exposure and human metabolism data. Four new metabolites of PCMC (hydroxylated, sulphated/hydroxylated, sulphated PCMC and PCMC glucuronide) were identified using the in vitro HLM/S9 assay. But only one metabolite, sulphated PCMC, was confirmed in wastewater and in urine. Therefore, our study confirms that water fingerprinting is a promising tool for biomarker selection and that *in vitro* HLM/S9 studies alone, although informative, do not provide high accuracy results. Our work also confirms, for the first time, human internal exposure to PCMC.

3.3 Introduction

3.3.1 Antimicrobials in personal care products and public exposure

Antimicrobials are extensively used as additives in a broad range of personal care and consumer products to preserve the integrity of the products against biological agents, although their effectiveness against the potential hazard has been questioned(Aiello et al. 2007). In particular, antimicrobials are added to soaps, cosmetics and disinfectants to protect against the growth of microorganisms, including bacteria, viruses and fungi. Some of these chemicals, their metabolites and/or their degradation products have been reported to be potentially bioaccumulative(Dhillon et al. 2015), endocrine disrupting(Ahn et al. 2008), ecotoxic in aquatic ecosystems(Rostkowski et al. 2011) and leading to microbial resistance(Aiello et al. 2005; Gautam et al. 2014). However very little is known about actual human exposure to antimicrobials in personal care products and therefore about the possibility to cause long term health effects. Even though available information concerning the percutaneous absorption of antimicrobials in humans is still scarce, it is known that some of them can be absorbed through the skin(Moss et al. 2000), suggesting that exposure results mostly from topical application of personal care products. However, ingestion of contaminated food and water (Loraine and Pettigrove 2006; Wu et al. 2013) and inhalation of indoor dust(Geens et al. 2009) represent other important indirect/environmental sources of exposure. Antimicrobials can be metabolised in humans followed by excretion of parent compound and their metabolites primarily with urine. Because the presence of those compounds in blood, serum and urine has been demonstrated(Allmyr et al. 2008; Asimakopoulos et al. 2014; Heffernan et al. 2015; Wu et al. 2010; Ye et al. 2011) and their environmental persistence and widespread use documented, it is unsurprising that they can be found in wastewater and in the receiving environment(Coogan and La Point 2008; Kumar et al. 2014). Their omnipresence, potential for bioaccumulation and possible synergistic effects of mixtures have raised public concern regarding their possible effects on human health as well as their role in the development of antimicrobial resistance(Yazdankhah et al. 2006). There is therefore the need to consider a greater range of factors contributing to potential health effects of combined exposures within the risk assessment process. Risk assessment of mixtures is known to be difficult due to complexity of contributing factors when compared to the assessment of single chemicals(Silins and Högberg 2011). New approaches towards

risk assessment and evaluation of public exposure to antimicrobial agents in personal care products are therefore critically needed.

By comparing community levels of environmental stressors (both external and internal) with observed health effects, conclusions could be drawn as to whether elevated levels of certain chemicals could be linked with particular diseases. Such epidemiological studies are currently being undertaken via traditional approaches which use simple tools including case histories, questionnaires, or molecular epidemiology, which combines the above with sensitive laboratory techniques. These approaches monitor biological responses, rather than diseases in human populations through the usage of biomarkers(Chen et al. 2014). However, a limitation of molecular epidemiology, due to logistical difficulties and high cost, is the restricted size of study groups and inability to gather comprehensive information on the complexity of combined (and cumulative) exposure to mixtures of chemicals and their effects. Therefore the community lacks robust measures that can be used to gather real-time information on community-wide health.

3.3.2 Urban water fingerprinting for human metabolic biomarkers - a new approach in epidemiological human exposure studies

Urban water fingerprinting for human metabolic biomarkers is a new approach in epidemiological exposure studies that can revolutionise the way we estimate public exposure to chemicals. This approach is also known wastewater-based epidemiology (WBE). WBE is a new concept that aims to overcome the above limitations and to provide spatial and temporal near-real time estimation of community-wide exposure to wide range of chemicals. This unique approach assumes that epidemiological information can be retrieved from wastewater via the analysis of human metabolic biomarkers. Although still in its infancy, it is currently used to determine illicit drug use trends at the community level through the analysis of urinary biomarkers in wastewater(Baker et al. 2014; Daughton 2012a; Yang et al. 2016). This approach can be also extended to make a real time assessment of population health status(Reid and Thomas 2011). WBE postulates that specific human metabolic biomarkers (e.g. characteristic metabolites of toxicants or pollutants) excreted with urine and faeces, and resulting from exposure to certain chemicals, are pooled by the urban wastewater system providing evidence of the amount and type of toxicants or pollutants to which

a population contributing to the analysed water, has been exposed. Urban water fingerprinting can therefore provide anonymous and comprehensive estimation of the community-wide health status in near-real time.

3.3.3 Difficulties with identification of biomarkers of exposure in WBE

The selection of unique metabolic biomarkers that are characteristic for each individual chemical and route of exposure is a critical step in order to verify public exposure to these chemicals via WBE, e.g. in order to distinguish between internal and external exposure, and to account for direct disposal, since many sources contribute to chemicals being discharged into wastewater. Unfortunately, in the case of many chemicals, especially those that are not intended for human consumption (e.g. antimicrobials), there is no public knowledge of characteristic metabolic biomarkers that could be utilised in WBE. Nevertheless, due to their extensive use in personal care and consumer products(Schebb et al. 2011) dermal absorption is considered to be one of the main routes of human exposure. Understanding toxicokinetic process, including metabolism, is therefore crucial in the determination of toxicological effects and potential for bioaccumulation of these chemicals, as well as in the identification of biomarkers of exposure. Still, there are only a few studies which reported their *in vivo* or *in vitro* biotransformation. Wu, Liu and Cai (2010)(Wu et al. 2010) investigated the metabolism of triclosan *in vivo* and *in vitro.* They observed both oxidative and phase II metabolites and identified glucuronidated triclosan as the major metabolite. Schebb et al. (2011)(Schebb et al. 2011) reported that the 0.6% circa of the amount of triclocarban present in bar soaps (70 ± 15 mg) was absorbed through the skin and that the 25% of total amount was excreted in urine almost exclusively as N-glucuronides. Unfortunately, most antimicrobials still remain hardly investigated.

3.3.4 Objectives

We are proposing a novel conceptual framework for identification of metabolic biomarkers via combined human metabolism and urban water fingerprinting assays. In this study, we identified, for the first time, human specific metabolites of the antimicrobial agent, 4-chloro-3-methylphenol (PCMC), as potential biomarkers of community-wide exposure to PCMC via WBE. This antimicrobial agent, also known as 4-chloro-*m*-cresol, is a phenolic compound that has been proven to have an

estrogenic activity determined by an *in vitro* yeast bioassay(Miller et al. 2001). PCMC is also known to have an effect on Ca^{2+} homeostasis being a strong activator of the ryanodine receptors in the endoplasmic reticulum(Ortopedico et al.) and to interfere with the thyroid hormone functions(Ghisari and Bonefeld-Jorgensen 2009). To the authors' knowledge, there is no published data on metabolic pathways of PCMC in humans.

3.4 Experimental section

3.4.1 Reagents and analytical standards

Pooled human liver microsomes (HLM), S9 fraction pooled from human liver, β-nicotinamide adenine dinucleotide 2′-phosphate reduced (β-NADPH ≥ 95%), Uridine 5′-diphosphoglucuronic acid trisodium salt (UDPGA 98-100%), alamethicin from *Trichoderma viride* (≥ 98%), 3′-phosphoadenosine 5′-phosphosulphate lithium salt (PAPS ≥ 60%), 4-chloro-3-methylphenol (p-chlorocresol), potassium phosphate monobasic tetrasodium salt hydrate (KH_2PO_4), magnesium chloride hexahydrate ($MgCl_2$), were purchased from Sigma-Aldrich (Gilligam,UK). The internal standard: 4-chloro-3-methylphenol-2,6-d2, was purchased from QMX Laboratories Ltd.

Solvents were of HPLC purity and were purchased from Sigma-Aldrich (Gilligam, UK). Stock standard solutions were prepared in methanol and stored in the dark at -20°C. 24h volume-proportional (100 mL every 15 minutes) composite wastewater influent samples were collected in PTFE bottles from a local wastewater treatment plant (WWTP) serving 70000 inhabitants on the 8th of June 2015. They were then transported to the laboratory in cool boxes packed with ice blocks and filtered through GF/F 0.7 µm glass fibre filter (Whatman, UK).

3.4.2 In vitro assays for verification of metabolic profile of PCMC in humans

Two *in vitro* assays were selected in this study: HLM and combined HLM and S9 fraction. Currently HLM represents the most commonly used *in vitro* model, providing an affordable way to give a good indication of the cytochrome P450 (CYP) and uridine 5'-diphospho-glucuronosyltransferase (UGT) metabolic profile (Ballesteros-Gómez et al. 2014). Unfortunately, the absence of other enzymes such as N-acetyltransferase (NAT), glutathione S-transferase (GST) and sulphotransferase (ST) implies, as a result, an incomplete range of metabolites being formed. A valid alternative to the use of HLM is the liver S9 fraction which contains both microsomal and cytosolic

fractions (phase I and phase II metabolic enzymes) that lead to the formation of a range of metabolites giving, as a result, more representative metabolic profile when compared to HLM only. However, the overall amount of metabolites formed is lower due to lower enzyme activity in the S9 fraction when compared to microsomes. This might result in minor metabolites to remain unnoticed(Brandon et al. 2003). Therefore, in this paper, method development included different subcellular fractions (HLM and a combination of HLM and S9 fraction).

3.4.3 In vitro HLM assay for verification of metabolic profile of PCMC

10 µL of a phosphate buffer (50mM KH_2PO_4, pH 7.4, 5mM $MgCl_2$), 10 µL of analyte solution (50 µM) were mixed with 10 µL human liver microsomes spiked with 1 µL of an alamethicin solution 12.5 mg/mL and 10 µL of a 100µM UDPGA solution. The reaction was initiated by addition of 10 µL of a 10 mM NADPH solution followed by incubation at 37°C for 1.5 h. After 1.5 h of incubation 10 µL of a 100µM PAPS solution were added and the incubation continued under the same conditions for 1.5 h. The negative controls with either no analyte or no HLM were incubated as described above to exclude all the non-enzymatic reactions. Each specific incubation was performed in duplicate. The reaction was quenched with 100 µL of acetonitrile ice cold, followed by centrifugation at 10000 rpm for 10 min (Centrifuge 5418, Eppendorf). The supernatant was removed and transferred to a new eppendorf tube and gently dried down by a stream of nitrogen at 40°C using TurboVap evaporator (Caliper, UK). The resulting residue was reconstituted with 50 µL of a 80:20 H_2O:MeOH solution containing the internal standard (100 ng/mLM) and transferred into a polypropylene vial for analysis.

All analyses were undertaken using a Dionex Ultimate 3000 HPLC (Thermo Fisher UK Ltd.) coupled with a Bruker Maxis HD Q-TOF (Bruker) equipped with an electrospray ionization source. Nitrogen was used as nebulising gas at a flow rate of 11 L/min at a temperature of 220°C and at a pressure of 3 Bar. Capillary voltage was set at 4500 V and End Plate offset was set at 500 V. The analyses were performed in both positive and negative modes and acquisition was performed in both full scan mode (MS) and broadband CID acquisition mode (MS/MS). HyStar™ Bruker was used to coordinate the LC-MS system. Chromatographic separation of the metabolites formed was achieved by using a WATERS ACQUITY UPLC BEH C18 column (50

mm x 2.1 mm, 1.7 µm) and the following mobile phase composition: 1 mM ammonium fluoride in water (A) and methanol (B). The gradient elution both in ESI positive and negative mode was as follows: 5% B (0 -3 min) - 60% B (3 - 4 min) - 60% B (4 -14 min), - 98% B (14 - 17 min) - 5% (17.1 - 20 min). The flow rate was kept constant at 0.4 ml/min and the column temperature was set at 40 °C. The source and operating parameters were optimized as follows: capillary voltage, 4500 V; dry gas temperature, 220 ^{o}C (N_2); dry gas flow 12 L h^{-1} (N_2); quadrupole collision energy, 4 eV; collision energy, 7 eV MS (full-scan analysis) and 20 eV MS/MS (bbCID mode). Nitrogen was used as the nebulising, desolvation and collision gas. The method was fully quantitatively validated for PCMC (intra-day, accuracy 120.2%, precision 2.4%; inter-day, accuracy 120.2%, precision 3.5%; IQL, 22 ng/L; IDL, 6.6 ng/L; linearity range, 0.07-27.5 mg/mL; R^2 0.9987; MDL, 0.013 ng/L; MQL, 0.045 ng/L).

3.4.4 In vitro combined HLM/S9 fraction assay for verification of metabolic profile of PCMC

Two incubation mixtures were prepared in duplicate by mixing 10 µL of phosphate buffer (50mM KH_2PO_4, pH 7.4, 5mM $MgCl_2$), 10 µL of analyte solution (50µM), 10 µL of the100µM UDPGA solution and 10 µL of HLM spiked with 1 µL of an alamethicin solution 12.5 mg/mL. The reaction was initiated by addition of 10 µL of a 10 mM NADPH solution followed by incubation at 37°C. The incubation was carried out for 3 h under the same conditions for three of the four samples. At 3 h 10 µL of S9 fraction and 10 µL the 100µM PAPS solution were added to the samples to be incubated for six h and incubation was continued. The negative controls with either no analyte or no enzymes were prepared as well for each time point. After quenching the reaction with 100 µL of acetonitrile ice cold, samples were prepared for analysis as described above.

3.4.5 In vivo pooled urine assay

Seven pooled urine samples were collected from a UK festival event. They came from five different urinals sampled on three different days. Solid phase extraction (SPE) was performed on pooled urine samples using HLB Oasis® cartridges Water, UK) to reduce the matrix effect and to concentrate each sample by 4-fold. SPE procedure was as follows: 2 mL of pooled urine were loaded onto Oasis HLB cartridges, which were preconditioned with 2 mL MeOH followed by 2 mL H_2O. After loading, the cartridges

were dried for 30 min and analytes were eluted with 4 mL MeOH. Extracts were then dried under a gentle nitrogen stream using a TurboVap evaporator (Caliper, UK, 40◦C). Dry extract was then reconstituted in 500 µL 80:20 H_2O:MeOH, transferred to polypropylene vials and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described above.

3.4.6 Wastewater fingerprinting assay

Raw wastewater samples collected from local wastewater treatment works, were filtered using GF/F glass microfibre filter 0.75 µm (Fisher Scientific, UK) followed by a solid phase extraction (SPE) using HLB Oasis® cartridges Water, UK) to reduce the matrix effect and to concentrate each sample by 400-fold. SPE procedure was as follows: 100 mL of filtered wastewater were loaded onto Oasis HLB cartridges, which were preconditioned with 2 mL MeOH followed by 2 mL H_2O. After loading, the cartridges were dried for 30 min and analytes were eluted with 4 mL MeOH. Extracts were then dried under a gentle nitrogen stream using a TurboVap evaporator (Caliper, UK, 40◦C). Dry extract was then reconstituted in 250 µL 80:20 H_2O:MeOH, transferred to polypropylene vials and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described above.

After analysis, data extracted from the Bruker system were processed with MetID software (Advanced Chemistry Development, Inc., ACD/Labs, UK) in order to predict metabolite structures. However, the software predicts a large number of possible metabolites, of which a rather small number is actually observed in *in vitro* experiments. We therefore developed a systematic workflow as presented in Figure 3.1 to limit false positive measurements.

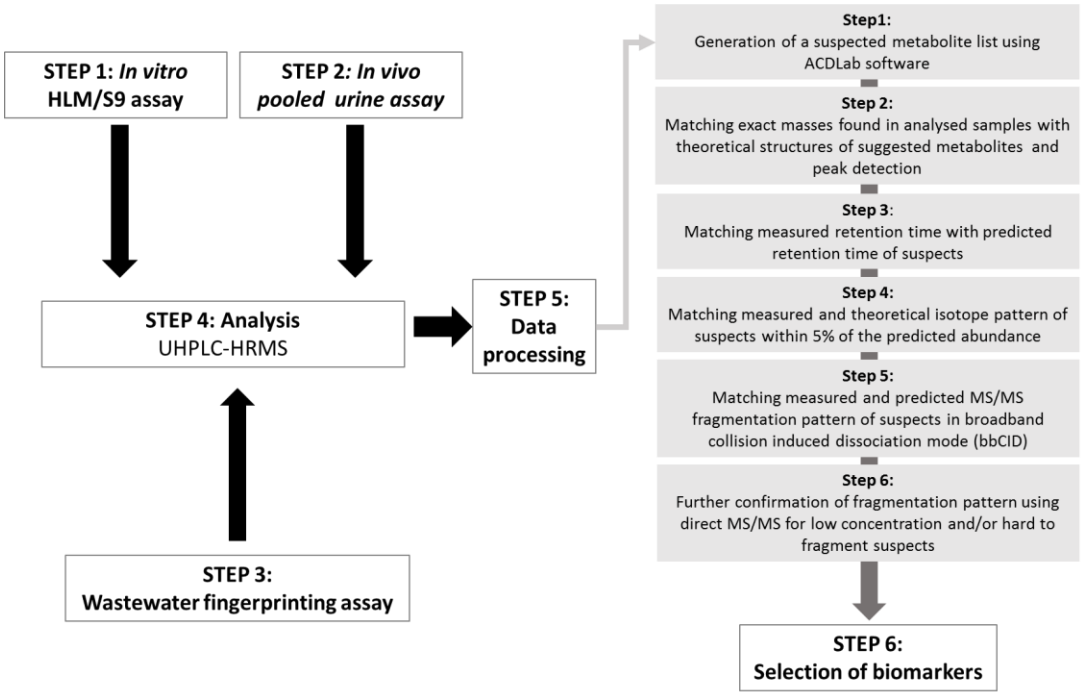


Figure 3.1 A systematic workflow for verifying human exposure to chemicals via combined in-vitro HLM/S9 and in-vivo pooled urine and wastewater profiling assay

3.5 Results and discussion

3.5.1 In vitro assays

The *in vitro* metabolism of PCMC catalysed by CYP and SULT enzymes has been investigated using a combination of pooled HLM an S9 fraction tests. Hydroxylation of un-substituted carbon atoms was expected to be the major biotrasformation reaction catalysed by CYPs whilst conjugations with phase II cofactors were expected to be the major reactions catalysed by UGT and ST. Phase II conjugations were expected to occur directly or following mono- and/or di-hydroxylation phase-I biotransformations.

3.5.2 In vitro HLM assay

After incubating PCMC with HLM a number of peaks were detected using LCMS. Initial analysis of samples, performed using ACDLabs software, identified two potential metabolites. A representative extracted ion chromatogram (XIC) of PCMC metabolites detected are reported in Figs. S3.1 and S3.2. All samples were analysed in negative and in positive ionisation modes. However, all the potential metabolites had better intensity in the negative ionization mode.

Incubation of PCMC produced a metabolite (m/z 157.0057) with elemental composition of the deprotonated molecule denoting C7H6ClO- (-3.6 ppm mass error) and a second one (m/z 317.0422) with elemental composition of the deprotonated molecule denoting C13H14ClO7- (-3.8 ppm mass error). ACDLabs analysis led to their identification as mono-hydroxylated metabolite (Fig S3.1b) and glucuronide conjugated (Fig. S3.2b). PCMC hydroxylate did not provide a distinctive fragmentation pattern in bbCID mode which necessitated MS/MS analysis. Fragmentation of ions with m/z 157.0062 +/- 0.005 at 31 eV led to the formation of a fragment 121.0284 which corresponded with the loss of a chlorine moiety from the precursor ion (Fig. S3.1c). PCMC glucoronate instead produced in bbCID mode a fragment ion at m/z 141.0108 (C7H6ClO-, + 3.5 ppm mass error) that was assigned to [$C_6H_8O_6$] loss, and was related to the presence of a glucuronate group (Fig. S3.2c, bottom). The fragments obtained confirmed the chemical structure of the metabolites. Additionally, two chlorine isotope peaks at m/z 158.0086 and m/z 159.0024 (Fig. S3.1d) and at m/z 318.0452 and m/z 319.0390 (Fig. S3.2d) were observed. The peaks had small mass errors (<5 ppm) and their relative heights match those expected from a compound with one chlorine within 5% of the predicted abundance.

PCMC metabolites have not been previously documented in literature, therefore the results of this study are of considerate importance. However, sulphate metabolites that were initially thought to be suitable as a biomarker were not detected in the *in-vitro* HLM assay. This could be due to two main factors. Firstly, the incubation time may not have been sufficiently long to allow detectable amounts of metabolites to be formed, as well as also not allowing the higher number of metabolites to be produced. Secondly this could be due to the lack of phase II enzymes being used such as sulphotransferases, of which HLM are deficient. To account for this, HLM/S9 fraction assay was undertaken (see below).

3.5.3 In vitro combined HLM/S9 fraction assay

The *in vitro* combined HLM/S9 fraction assay included verification of quantitative and qualitative changes of metabolic profile in two time intervals (3 and 6 h). Moreover, due to the addition of the S9 fraction to the incubation mixture, further metabolites (sulphate conjugated) were expected to be produced. Indeed, an

incubation of PCMC with pooled HLM/S9 fraction produced two further metabolites: sulphated PCMC and mono-hydroxylated sulphated PCMC (Fig. 3.2 and S3.3).

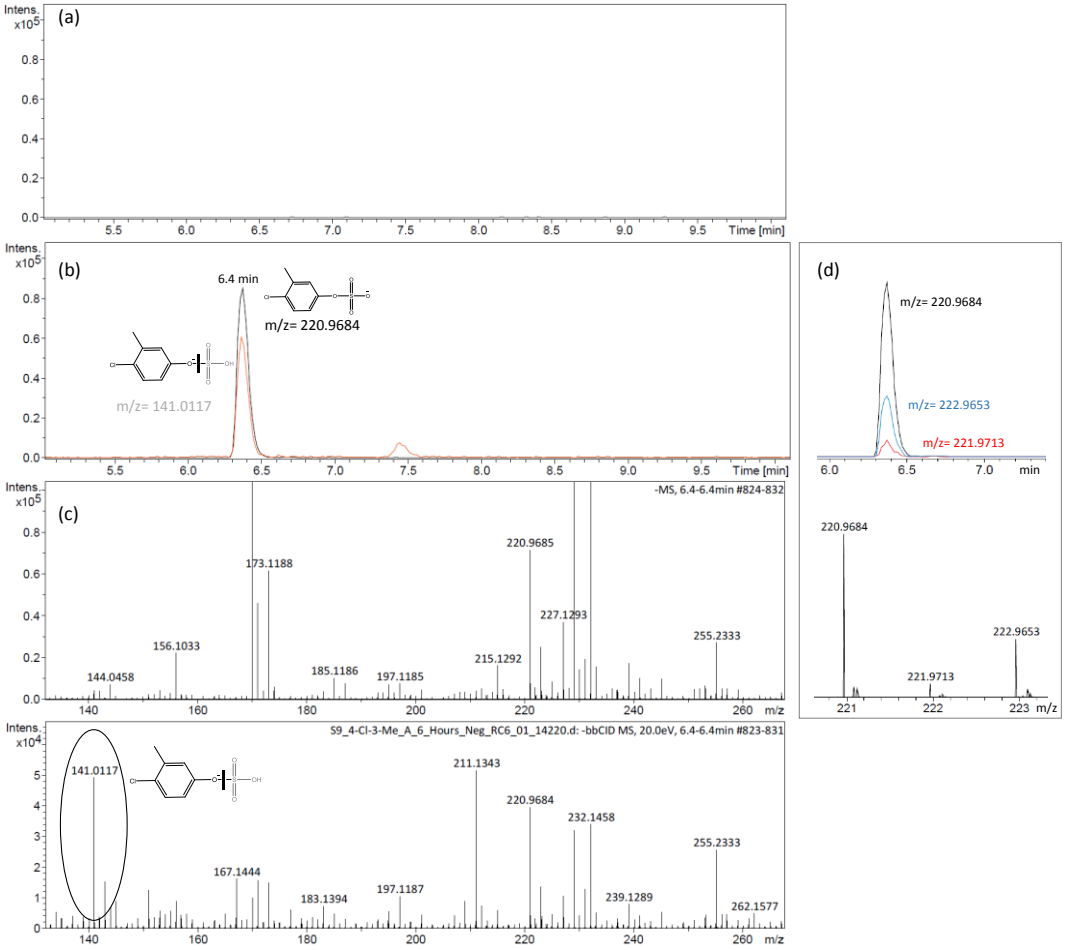


Figure 3.2 Detection and identification of sulphated PCMC by UHPLC-QTOF-MS following in-vitro HLM/S9 assay. XICs at m/z 220.9681 and 141.0113 (0.005-Da mass-window width) for analyte-sample (b) and control-sample (a). (c) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed. (d) XIC at m/z 220.9684, 221.9713 and 222.9653 for PCMC sulphate and the two chlorine isotope peaks (top) and mass spectra (bottom).

It can be seen in Fig. 3.2 that the *in vitro* test leads to the formation of a metabolite with retention time denoting 6.4 min (Fig. 3.2b, dark peak). This chromatographic peak was absent in the blank control (Fig. 3.2a). Spectral analysis performed using ACDLabs software identified the compound to be a sulphated metabolite (m/z 220.9684). Elemental composition of the deprotonated molecule of the sulphated metabolite was assigned as C7H6ClO4S- (+ 1.3 ppm mass error). The fragment ion at m/z 141.0117 (C7H6ClO-, + 3.6 ppm mass error) was assigned to [O_3S] loss, and was

related to the presence of a sulphate group (Fig. 3.2c, bottom). To further confirm that the fragment ion originates from the suspected metabolite its chromatogram was extracted. The resulting XIC produced a peak whose elution time matched perfectly with that of the suspected metabolite (Fig. 3.2b, light peak). Additionally, the presence of two chlorine isotope peaks at m/z 221.9713 and m/z 222.9653 (Fig. 3.2d) was observed. The peaks had small mass errors <5 ppm and their relative heights match those expected from a compound with one chlorine within 5% of the predicted abundance.

The *in vitro* HLM/S9 fraction assay led to the formation of another PCMC metabolite with retention time of 6.3 min (Fig S3.3b, dark peak). This is the same chromatographic peak that was absent in the blank control (Fig. S3.3a). Spectral analysis performed using ACDLabs software identified the compound to be the sulphated and hydroxylated metabolite (m/z 236.9632). Elemental composition of the deprotonated molecule of the metabolite was assigned as C7H6ClO5S- (+ 1.3 ppm mass error). The fragment ion at m/z 157.0065 (C7H6ClO2-, + 1.9 ppm mass error) was assigned to [O_3S] loss, and was related, as previously, to the presence of a sulphate group (Fig. S3.3c, bottom). To further confirm that the fragment ion originates from the suspected metabolite its chromatogram was extracted. The resulting XIC produced a peak whose elution time matched perfectly with that of the suspected metabolite (Fig. S3.3b, light peak). Also, as above, two chlorine isotope peaks at m/z 237.9664 and m/z 238.9601 (Fig. S3.3d) were observed. The peaks had small mass errors <5 ppm and their relative heights matched those expected from a compound with one chlorine within 5% of the predicted abundance.

Phase II cofactor (PAPS) was added after 3 h to the incubation mixture to permit all the possible phase I metabolites to form before conjugation with sulphate took place. This approach attempts to replicate what happens in a living cell, where generally (but not necessarily) phase I minor biotransformations occur in preparation for successive phase II conjugation. Results are summarised in Fig. S3.4. It can be seen from Fig. S3.4 that hydroxylated metabolites are preferentially formed after 3 h of incubation time (88.7% against 11.3% conjugation with glucuronic acid). The hydroxylated PCMC was still the most abundant biotransformation product (40% of the total metabolites produced circa) after 6 h of incubation time, although at this sampling

point phase II metabolites accounted for 59.8% of all the metabolites produced. In particular amongst the three phase II biotransformation observed after 6 h direct sulphation seemed to be the preferential conjugation route accounting for more than 25% of total biotransformation.

In summary, both HLM and HLM-S9 fraction assays allowed for the identification of metabolites that have not been previously documented in literature, although the latter assay allowed the identification of a higher number of metabolites due to the addition of the S9 fraction resulting in a more efficient sulphation. Moreover a two-step approach, which entails the addition of phase II enzymes and sulphation cofactor after 3 h permits the identification of all the phase I and II metabolites and conjugated metabolites, providing a wider range of biotransformation products. The formation of PCMC sulphate conjugates means also that a more efficient sulphate conjugation takes place in the HLM-S9 fraction assay, when compared to the HLM assay. All the identified metabolites are presented in Tab. 3.1. The table reports also elemental composition and the mass accuracy measured in the two *in vitro* assays and in a wastewater sample from a local wastewater treatment plant (WWTP) (see discussion below).

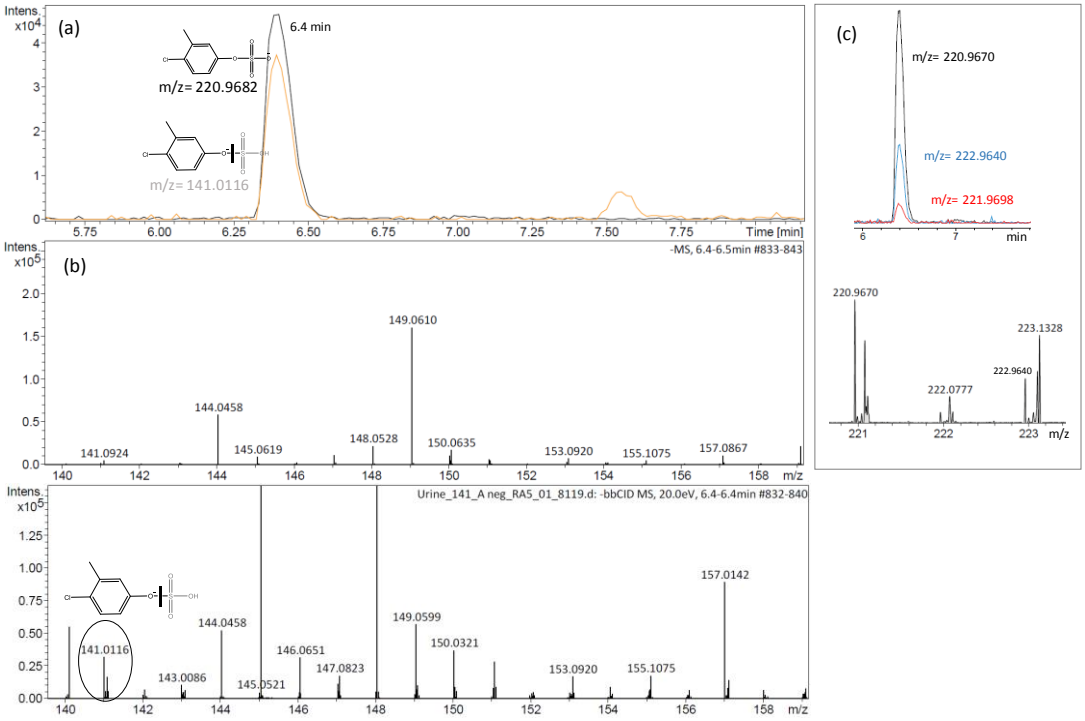


Figure 3.3 Detection and identification of sulphated PCMC by UHPLC-QTOF-MS following in-vivo poled urine assay. (a) XICs at m/z 220.9681 and 141.0113 (0.005-Da mass-window width). (b) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed. (c) XIC at m/z 220.9670, 221.9698 and 222.9640 (0.005-Da mass-window width) for PCMC and the two chlorine isotope peaks (top) and mass spectra (bottom).

3.5.4 In vivo pooled urine assay

The *in vivo* pooled urine assay led to identification of only one metabolite of PCMC, sulphated PCMC (Tab. 3.1 and Fig. 3.3). Interestingly, hydroxylated and glucuronidated metabolites were not observed in analysed pooled urine samples. This is in contrast with *in vitro* assays where glucuronidated, sulphated and hydroxylated metabolites were identified.

3.5.5 In vivo wastewater fingerprinting assay

The aim of the two *in vitro* assays was to select potential biomarkers of exposure to PCMC. However, as the ultimate goal of this study was to verify community-wide exposure to these chemicals, analysis of untreated wastewater samples serving large community of 70 thousand people was undertaken. The identification of biomarkers was based on the systematic workflow presented in Fig. 3.1. The compounds detected in wastewater are summarised in Tab. 3.1. As expected, given the complexity of the matrix, mass accuracy measured was lower than that measured

in *in vitro* studies but still within set limits, with mass error values between 5 and 10 ppm (Tab. 3.1).

Table 3.3.1 PCMC and their metabolic biomarkers.

			In-vitro HLM assay		In-vitro HLM/S9 fraction assay		In-vivo pooled urine assay		In-vivo wastewater fingerprinting assay	
Compound	**Elemental composition [M-H]$^-$**	**Exact mass (m/z)**	**Peak top mass (m/z)**	**Mass error (ppm)**	**Peak top mass (m/z)**	**Mass error**	**Peak top mass (m/z)**	**Mass error**	**Peak top mass (m/z)**	**Mass error (ppm)**
PCMC	$C_7H_6ClO^-$	141.0113	141.0118	+3.6	141.0116	+2.1	-	-	141.0122	+6.0
PCMC hydroxylated	$C_7H_6ClO_2^-$	157.0062	157.0049	-8.2	157.0061	-0.6	-	-	-	-
PCMC glucuronidated	$C_{13}H_{15}ClO_7^-$	317.0434	317.0422	-3.8	317.0442	+2.5	-	-	-	-
PCMC sulphated	$C_7H_6ClO_4S^-$	220.9681	-	-	220.9684	+1.3	220.9670	- 5	220.9695	+6.4
PCMC hydroxylated & sulphated	$C_7H_6ClO_5S^-$	236.9630	-	-	236.9632	+0.9	-	-	-	-

In vivo wastewater fingerprinting assay resulted in the detection and identification of only one metabolite of PCMC, sulphated PCMC, in wastewater (Fig. 3.4). The loss of [O_3S] deduced by TOF MS spectra has been crucial for justifying and suggesting possible chemical structures. Interestingly, hydroxylated and glucuronidated PCMC were not observed in analysed wastewater samples. This is in line with results obtained for *in vivo* pooled urine assay and it confirms that *in vitro* studies, although informative, cannot serve as the only tool intended for selection of biomarkers of exposure.

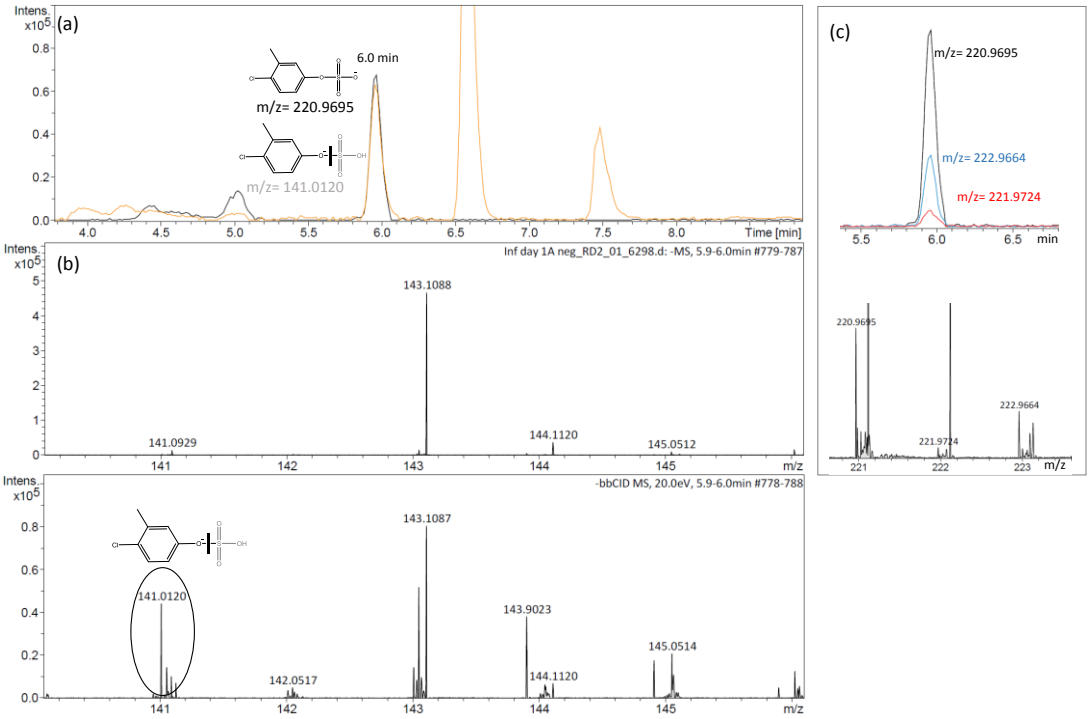


Figure 3.4 Detection and identification of sulphated PCMC by UHPLC-QTOF-MS following in-vivo wastewater profiling assay. (a) XICs at m/z 220.9681 and 141.0113 (0.005-Da mass-window width). (b) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed. (c) XIC at m/z 220.9695, 221.9724 and 222.9664 (0.005-Da mass-window width) for PCMC and the two chlorine isotope peaks (top) and mass spectra (bottom).

3.6 Conclusions

This study proved that combined human metabolism and wastewater fingerprinting assay is a powerful tool to investigate human exposure to chemicals present in personal care products and a wider-group of chemicals that are not intended for human consumption and therefore lack comprehensive risk assessment data. We have proposed a systematic workflow that enables fast and comprehensive selection of characteristic biomarkers of public exposure to chemical substances (Fig. 3.1). The workflow consists of several steps: Step 1: *In vitro* HLM/S9 assay; Step 2: *In vivo* pooled urine assay; Step 3: *In vivo* wastewater fingerprinting assay; Step 4: Analysis with HR-MSMS; Step 5: Data processing and Step 6: Selection of biomarkers. In Step 4, after the establishment of a list of suspected metabolites using ACDLab software (Step 4a), in order to avoid false positives, their accurate mass, retention time and fragmentation pattern are examined (Step 4b,c,d). Finally the structure of the suspects is confirmed by investigating the MS/MS fragmentation pattern in bbCID mode (Step 4e). For those metabolites that do not provide an optimal MS/MS fragmentation pattern in bbCID mode, a further confirmation step performing a data-dependent MS/MS acquisition is required (Step 4f), i.e. an MS/MS analysis is triggered if a compound from a target ion list is detected. In contrast to targeted screening, non-target screening starts without any a priori information on the compounds to be detected. However, this study falls in between these two categories, since the chemically meaningful structures which can be assigned to an unknown peak are limited to structures showing a close relationship with the parent compound.

Four new possible metabolites of PCMC (hydroxylated, glucuronidated, sulphated and hydroxylated & sulphated PCMC) were identified after *in vitro* HLM/S9 studies and were proposed as biomarkers of exposure. The absence of phase I metabolites in the presence of phase II cofactor PAPS suggested that sulphation was the preferential metabolic pathway for this compound. Only one of these metabolites (PCMC sulphated) was confirmed in wastewater and in urine suggesting human internal exposure to PCMC despite the fact that this compound is utilised in products meant for external use. Consequently to the results obtained in this present work it seems evident that the impact of the exposure to PCMC and other chemicals not intended for human consumption might need to be reconsidered. Also in a realistic overview of its

impact on the aquatic ecosystem its identified metabolite should be also investigated to verify their potential environmental impact.

The aim of this paper was to introduce a new assay for identification of new metabolic biomarkers in WBE. Further work will be undertaken to verify utility of selected biomarkers in a large urban water catchment monitoring campaign.

3.7 Acknowledgements

The support of the Leverhulme Trust (Project No RPG-2013-297) is greatly appreciated. We would also like to acknowledge TICTAC Communications (St George's University of London, United Kingdom) for provision of pooled urine samples. All data supporting this study are provided as supporting information accompanying this paper.

3.8 Supplementary material

3.8.1 List of figures

Figure S3.1 XIC of hydroxylated PCMC metabolite produced with HLM. XICs at m/z 157.0062 (0.005-Da mass-window width) for analyte-sample (b), blank control (a), fragmentation pattern of the metabolite obtained in MRM mode (c) and XIC at m/z 157.0049, 158.0079 and 159.0017 for PCMC and the two chlorine isotope peaks (top), and mass spectra (bottom).

Figure S3.2 Detection and identification of PCMC glucuronate metabolite by UHPLC-QTOF-MS following in-vitro HLM assay (3 hour time point). XICs at m/z 317.0422 and 141.0110 (0.005-Da mass-window width) for analyte-sample (b) and control-sample (a). (c) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra of the metabolite and fragment ion observed. (d) XIC at m/z 317.0422, 318.0452 and 319.0390 for PCMC glucoronate and the two chlorine isotope peaks (top), and mass spectra (bottom).

Figure S3.3 Detection and identification of sulphated and hydroxylated PCMC by UHPLC-QTOF-MS following in-vitro HLM/S9 assay. XICs at m/z 236.9630 and 157.0062 (0.005-Da mass-window width) for analyte-sample (b) and control-sample (a). (c) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed. (d) XIC at m/z 236.9632, 237.9660 and 238.9601 for PCMC hydroxylate & sulphate and the two chlorine isotope peaks (top) and mass spectra (bottom).

Figure S3.4 Distribution of PCMC metabolites obtained with in-vitro HLM and HLM/S9 fraction assay over a 3 and 6 h incubation time.

3.8.2 List of reports (appendix A)

(Available online)

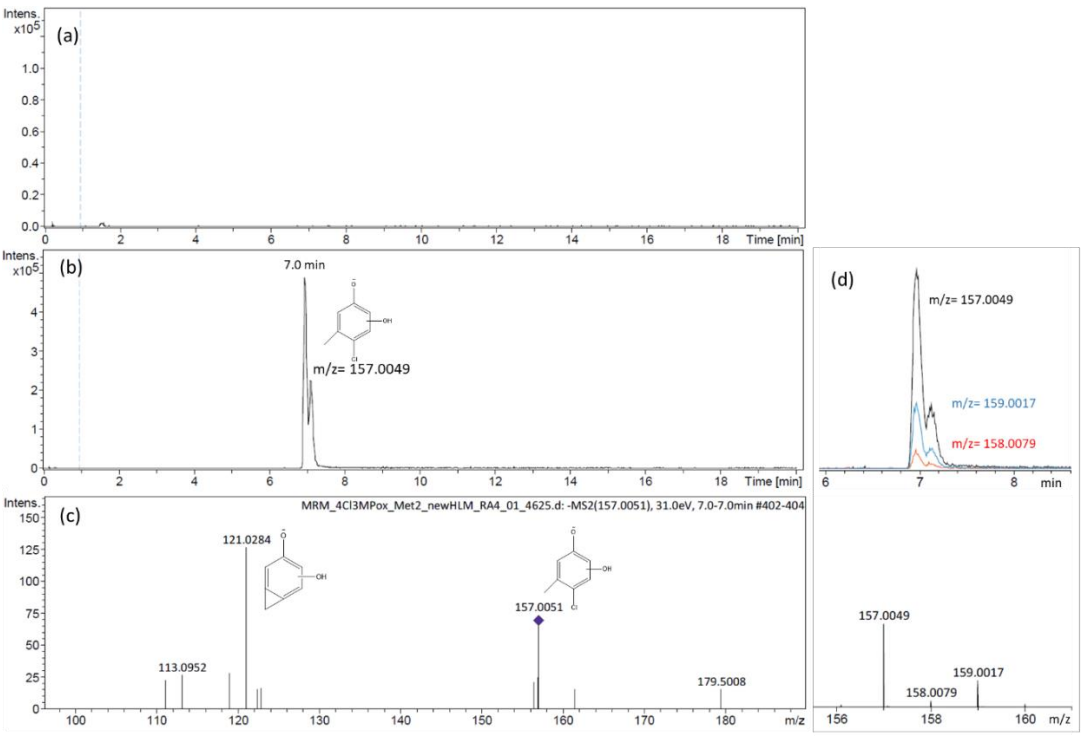


Figure S3.1 XIC of hydroxylated PCMC metabolite produced with HLM. XICs at m/z 157.0062 (0.005-Da mass-window width) for analyte-sample (b), blank control (a), fragmentation pattern of the metabolite obtained in MRM mode (c) and XIC at m/z 157.0049, 158.0079 and 159.0017 for PCMC and the two chlorine isotope peaks (top), and mass spectra (bottom).

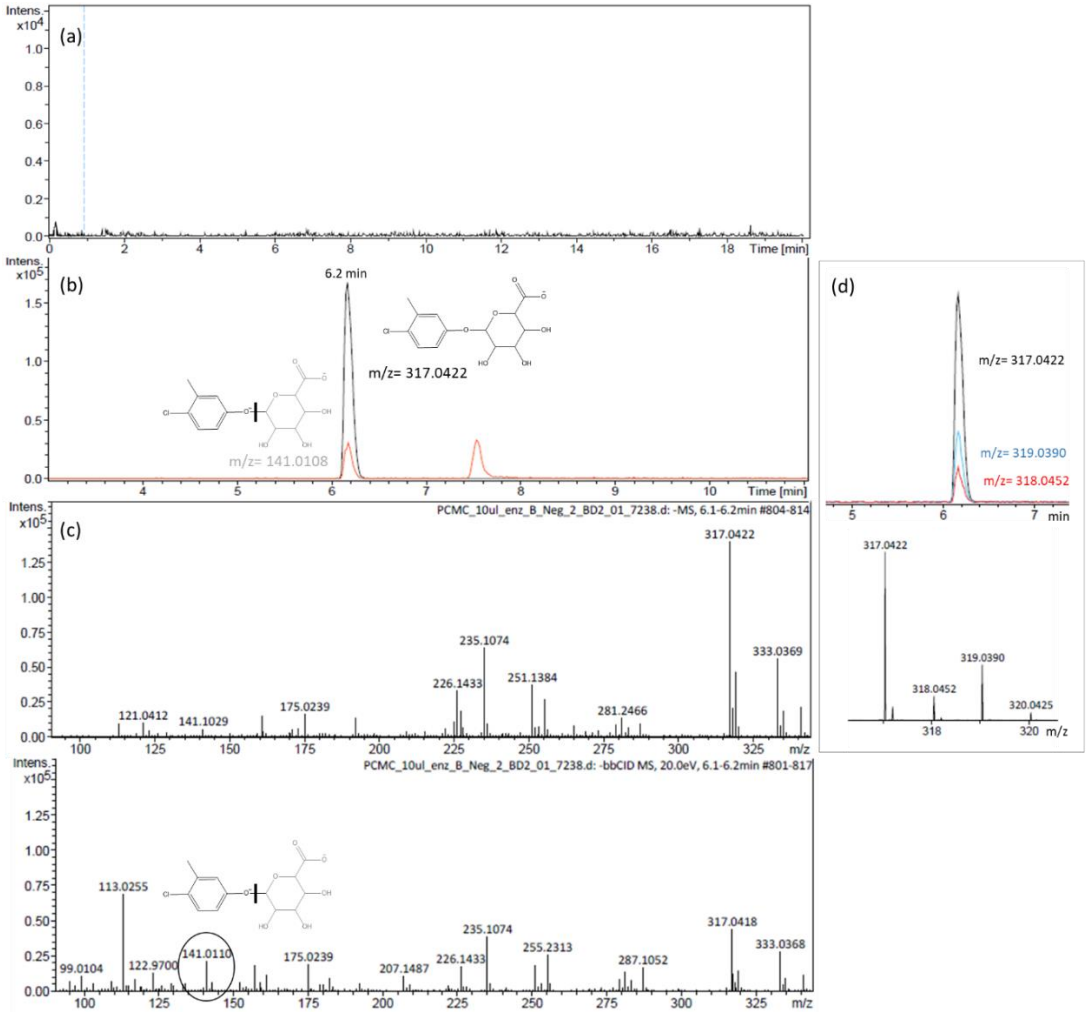


Figure S3.2 Detection and identification of PCMC glucuronate metabolite by UHPLC-QTOF- MS following in-vitro HLM assay (3 hour time point). XICs at m/z 317.0422 and 141.0110 (0.005-Da mass-window width) for analyte-sample (b) and control-sample (a). (c) (top) Low- energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra of the metabolite and fragment ion observed. (d) XIC at m/z 317.0422, 318.0452 and 319.0390 for PCMC glucoronate and the two chlorine isotope peaks (top), and mass spectra (bottom).

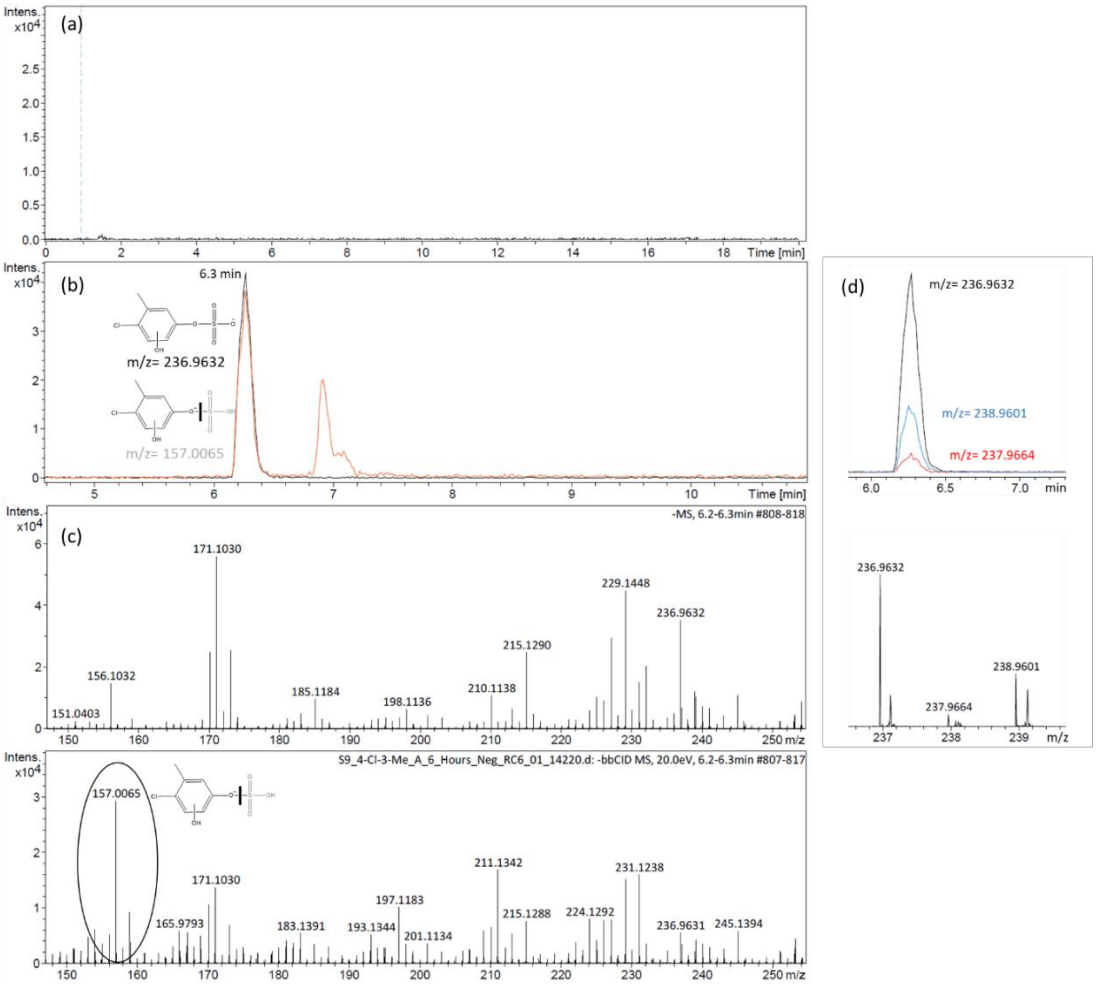


Figure S3.3 Detection and identification of sulphated and hydroxylated PCMC by UHPLC- QTOF-MS following in-vitro HLM/S9 assay. XICs at m/z 236.9630 and 157.0062 (0.005-Da mass-window width) for analyte-sample (b) and control-sample (a). (c) (top) Low-energy (full- scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed. (d) XIC at m/z 236.9632, 237.9660 and 238.9601 for PCMC hydroxylate & sulphate and the two chlorine isotope peaks (top) and mass spectra (bottom).

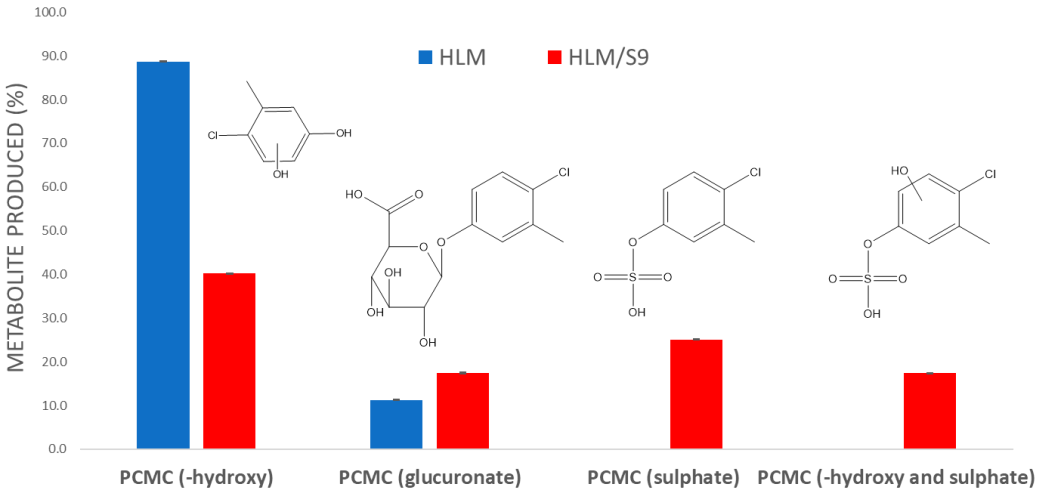


Figure S3.4 Distribution of PCMC metabolites obtained with in-vitro HLM and HLM/S9 fraction assay over a 3 and 6 hours incubation time.

3.9 References

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Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.
Signed	**Date**

4 Verifying community-wide exposure to endocrine disruptors in personal care products – in quest for metabolic biomarkers of exposure via in vitro studies and wastewater-based epidemiology

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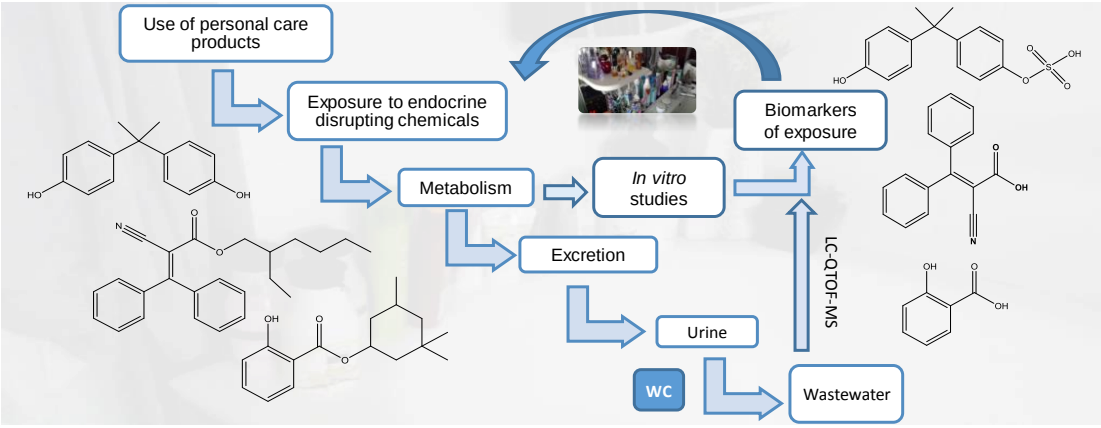
4.1 Graphical abstract



Keywords: wastewater, epidemiology, exposure, UV filter, endocrine disruptor, personal care product, environment

4.2 Abstract

This study aimed to identify human specific metabolites of selected known or suspected endocrine disruptors (EDCs), mainly UV filters, used in personal care and consumer products whose metabolism has hardly been explored and to select suitable candidate biomarkers for human exposure studies using wastewater-based epidemiology (WBE). The analysis of metabolic biomarkers of target chemicals is crucial in order to distinguish between internal and external exposure, since many sources contribute to chemicals being discharged into wastewater. This was achieved through the employment of a new analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting. Eight EDCs with unknown metabolic pathways (benzophenone-1 (BP-1); benzophenone-2 (BP-2); 4,4'-dihydroxybenzophenone (4,4'-DHBP); 4-benzylphenol (4-BenzPh); homosalate (HO); octocrylene (OC); 3-benzylidene camphor (3-BC), and two EDCs with known metabolism (bisphenol A (BPA) and benzophenone-3 (BP-3)) were tested. The biotrasformation observed consisted mainly in phase I processes such as hydrolysis and hydroxylation together with phase II conjugation reactions such as sulphation and glucuronidation. Only two chemicals (BP-1, BP-3) were identified in urine and three chemicals (BPA, BP-1, BP-3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in tested urine/wastewater samples from five WWTPs serving large communities ranging between 17 and 100 thousand inhabitants. The presence of metabolic biotransformation products of OC, 4-BenzPh, BPA and HO in wastewater provides evidence for internal exposure of studied populations to these chemicals.

4.3 Introduction

The last decades have seen a rapid increase in usage of personal care products and resultant public exposure to chemicals contained in those products (Calafat et al. 2015). Although the level of exposure to many of these chemicals might be low, the risk associated with it cannot be underestimated since simultaneous exposure to undefined mixtures might result in a synergistic effect making a comprehensive risk assessment process more complex compared to the assessment of a single chemical (Silins and Högberg 2011). Long-term exposure leading to chronic effects should also not be underestimated. However very little is known about actual human exposure and therefore about the possibility to cause long term effects. UV filters are extensively used in a wide range of products including plastics, adhesives, rubber and personal care and consumer products including cosmetics, body lotions, hair sprays, skin creams, hair dyes or shampoos to preserve the integrity of the products (or skin, in the case of cosmetic sunscreens) from damage caused by the ultra-violet (UV) component of sunlight (particularly UVB, 290–320 nm). Some of these chemicals, their metabolites and/or their degradation products have been reported to be potentially bioaccumulative (Environment Agency 2008), leading to endocrine disrupting effects(Krause et al. 2012; Zhao et al. 2013) and ecotoxicity in aquatic ecosystems (Díaz-Cruz and Barceló 2009; Fent et al. 2008; Kaiser et al. 2012). In particular homosalate (HO), ethylhexylmethoxycinnamate (EHMC), octocrylene (OC) and benzophenone-3 (BP-3, regulated in the EU and commonly used in personal care products in the UK (Kerr 2011)), have been demonstrated to exert endocrine disrupting effects *in vitro* and/or *in vivo*. According to Schlumpf et al. (Schlumpf et al. 2001) HO showed estrogenic effects *in vitro* (median effective concentration (EC_{50}) at 1.56 µM) and but not *in vivo* at similar concentrations, whereas EHMC has been proven to interfere with multiple endocrine pathways in rats (EC_{50} 934 mg/kg/day). Seidlová–Wuttke et al. (Seidlová-Wuttke et al. 2006) observed a weak estrogenic effect of EHMC at high concentrations (275 mg/day for 6 weeks) while Klammer et al. (Klammer et al. 2007) observed an alteration of the hypothalamo-pituitary-thyroid function in the animals after 5 days of treatment. OC instead has been proven to exert antiestrogenic activity *in vitro* and *in vivo* in fish by Kunz et al., 2006 (Kunz and Fent 2006b). Additionally some of these compounds are capable of damaging DNA via formation of radicals (Inbaraj et al. 2002).

Even though available information concerning the percutaneous absorption of UV filters in humans is still scarce, it is known that some of them can be absorbed through the skin (León-González et al. 2013), suggesting that exposure results mostly from topical application of personal care products (Ko et al. 2016). Moreover, Schlumpf et al. (Schlumpf et al. 2010) found a correlation between the presence of UV filters in human milk and the use of cosmetics, indicating that internal exposure resulted consistently from the usage of cosmetics. Ingestion of contaminated food and water (Loraine and Pettigrove 2006; Wu et al. 2013) and inhalation of indoor dust represent other important indirect/environmental sources of exposure (Geens et al. 2009; Wang et al. 2013). The presence of those compounds has been also demonstrated in urine (Asimakopoulos et al. 2014; Schauer et al. 2006b), wastewater and ultimately in the receiving environment as a consequence of their environmental persistence and widespread use (Gago-Ferrero et al. 2015; Gautam et al. 2014; Li et al. 2007; Poiger et al. 2004). Furthermore, given their physico-chemical properties, many of the compounds under investigation show great potential for bioaccumulation contributing to the formation of complex environmental mixtures raising public concern regarding their possible effects on human health and ecosystems (Balmer et al. 2005; Meyer et al. 2009). Dermal absorption of UV filters is considered to be one of the main routes of human exposure due to their extensive use in personal care and consumer products (León-González et al. 2013; Schauer et al. 2006b; Schebb et al. 2011). Understanding toxicokinetic process, including metabolism, is therefore crucial in the determination of toxicological effects and bioaccumulation of these chemicals, as well as in the identification of biomarkers of exposure. Still there are only a few studies which reported the *in vivo* or *in vitro* biotransformation of UV filters. The metabolism of benzophenone-type UV filters has been investigated by Jeon et al. (Jeon et al. 2008) in rats observing mainly oxidative metabolites. These results were in accord with Watanabe et al. (Watanabe et al. 2015) who observed oxidative metabolites of BP-3 *in vitro*. Phase I and phase II metabolites of 3-(4′-methylbenzylidene)camphor (4-MBC) were found in urine, after oral administration in rats, as well as after dermal administration in both humans and rats (León-González et al. 2013; Schauer et al. 2006b; Völkel et al. 2006). However, most other UV filters still remain hardly investigated.

This study aims to identify human specific metabolites of selected endocrine disruptors (EDCs), mainly UV filters, used in personal care and consumer products whose metabolism has hardly been explored and to select suitable candidate biomarkers of human exposure studies to these chemicals and for possible application in wastewater based epidemiology (WBE). The analysis of metabolic biomarkers of target chemicals is crucial in order to distinguish between internal and external exposure (e.g. direct disposal), since many sources contribute to chemicals being discharged into wastewater. This could be achieved through the employment of a new analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting that was proposed by Lopardo et al. (Lopardo et al. 2017). Table S 4.1 shows a list of eleven compounds that were prioritised in this study. The list includes nine chemicals (UV filters) used in personal care and consumer products, which are established or suspected EDCs and whose metabolism is yet unknown, and two compounds with known metabolism (bisphenol A (BPA) and benzophenone 3 (BP-3)).

4.4 Experimental section

4.1.1 Reagents and analytical standards.

Water and methanol were of HPLC purity level and were purchased from Sigma-Aldrich. Pooled human liver microsomes (HLM), S9 fraction pooled from human liver, β-nicotinamide adenine dinucleotide 2′-phosphate reduced (β-NADPH ≥95%), alamethicin from Trichoderma viride (≥98%), 3′-phosphoadenosine 5′-phosphosulphate lithium salt (PAPS, ≥60%), uridine 5′-diphosphoglucuronic acid trisodium salt (UDPGA, 98-100%), were purchased from Sigma-Aldrich (Gilligam, UK). The reference standards: 4-chloro-3-methylphenol (PCMC), 4-benzylphenol (4-BenzPh), bisphenol A (BPA), bisphenol A-D16, homosalate (HO), 3-benzylidene camphor (3BC), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), 4,4'-dihydroxybenzophenone (4,4'-DHBP), , octocrylene (OC), potassium phosphate monobasic tetrasodium salt hydrate (KH_2PO_4), magnesium chloride hexahydrate ($MgCl_2$), were purchased from Sigma-Aldrich (Gilligam,UK). The following internal standards: 4-chloro-3-methylphenol-2,6-d2, triclosan-d3, benzophenone 3-d5 and bezafibrate-d6 were purchased from QMX Laboratories Ltd.

4.1.2 In vitro assays

Two step *in vitro* assay developed by Lopardo et al.(Lopardo et al. 2017), was undertaken in this study employing HLM and S9 fraction. 4-Cl-3-methylphenol was selected as a positive control to evaluate the performance of the assay. Two incubation mixtures were prepared in duplicate by mixing 10 µL of phosphate buffer (50 mM KH_2PO_4, pH 7.4, 5 mM $MgCl_2$), 10 µL of analyte solution (50 µM), 10 µL of 100 µM UDPGA solution, and 10 µL of HLM spiked with 1 µL of an alamethicin solution (12.5 mg/mL). The reaction was initiated by the addition of 10 µL of a 10 mM NADPH solution followed by an incubation at 37 °C. The incubation was carried out for 3 hours under the same conditions for two of the four samples. At 3 hours, 10 µL of S9 fraction and 10 µL the 100 µM PAPS solution were added to the samples to be incubated for six hours and incubation was continued. The negative controls with either no analyte or no enzymes were prepared as well for each time point. The reaction was quenched with 100 µL of acetonitrile ice cold, followed by centrifugation at 10 000 rpm for 10 min (Centrifuge 5418, Eppendorf). The supernatant was removed and transferred to a new eppendorf tube and gently dried down by a stream of nitrogen at 40 °C using

TurboVap evaporator (Caliper, UK). The resulting residue was reconstituted with 50 µL of a solution 80:20 H_2O/MeOH containing the internal standard (100 ng/mL), transferred into a polypropylene vial and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below. The range of metabolites produced and their relative ratios were investigated and compared (Table S 4.22).

4.1.3 **In vivo pooled urine essay**

In vivo pooled urine essays were performed on seven pooled urine samples collected from a UK festival event. The samples were collected on three different days from five different urinals. Solid phase extraction (SPE) was performed using HLB Oasis® cartridges (Water, UK). For further details see Lopardo et al. (Lopardo et al. 2017). In addition HLB Oasis® cartridges, MCX and MAX Oasis® cartridges (Water, UK) were also used in this study. SPE extraction on the MCX and MAX cartridges was performed by loading 2 mL of pooled urine onto the cartridges, which were preconditioned with 2 mL MeOH (both cartridges) followed by 2 mL of H_2O with 2% formic acid on MCX and 2 mL of H_2O with 5% ammonium hydroxide on MAX. After loading, cartridges were dried for 30 min and elution was undertaken using 2 mL of MeOH (both cartridges), followed by 2 mL of MeOH with 7% ammonium hydroxide on MAX and 2mL of MeOH with 2% formic acid on MCX. Extracts were dried using a TurboVap evaporator (Caliper, UK,) under a gentle nitrogen stream in a water bath at 40°C then reconstituted in 500 µL 80:20 H_2O:MeOH and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below.

4.1.4 **Wastewater fingerprinting assay**

24h composite (flow proportional) samples of raw wastewater (after physical screening) were collected from 5 different wastewater treatment plants and filtered using GF/F glass microfibre filter 0.75 µm (Fisher Scientific, UK). Solid phase extraction (SPE) was performed using HLB (Lopardo et al., 2017), MCX and MAX Oasis® cartridges (Waters, UK). Extraction procedure included loading 100 mL of filtered wastewater onto Oasis cartridges which were preconditioned with 2 mL of MeOH for all cartridges followed by 2 mL of H_2O for HLB, 2 mL of H_2O with 2% formic acid for MCX and 2 mL of H_2O with 5% ammonium hydroxide for MAX.

After loading the cartridges were dried for 30 minutes and analytes were eluted with 2 mL of MeOH for all cartridges followed by 2 more mL of MeOH for HLB cartridges, 2 mL of MeOH with 7% ammonium hydroxide for MCX and 2 mL of MeOH with 2% formic acid for MAX. Extracts were dried using a TurboVap evaporator (Caliper, UK,) under a gentle nitrogen stream in a water bath at 40°C then reconstituted in 250 µL 80:20 H_2O:MeOH and analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to the procedure described below.

4.1.5 Liquid –chromatography coupled with tandem mass spectrometry

All samples were analysed with a Dionex Ultimate 3000 HPLC (Thermo Fisher UK Ltd.) coupled with a Bruker Maxis HD Q-TOF (Bruker) equipped with an electrospray ionization source. Nitrogen was used as nebulising gas at a flow rate of 11 L/min at a temperature of 220°C end at a pressure of 3 Bar. Capillary voltage was set at 4500 V and End Plate offset was set at 500 V. The method was applied both in ESI positive and negative and acquisition was performed in data independent broadband collision-induced dissociation acquisition mode (bbCID). In bbCID, precursor and product ion spectra were obtained by alternating low and high collision energy (respectively 7 and 20 eV). HyStar™ Bruker was used to coordinate the LC-MS system. Chromatographic separation of the metabolites formed was achieved by using an ACQUITY UPLC BEH C18 column (50 mm x 2.1 mm, 1.7 µm) and the following mobile phase composition: 1 mM ammonium fluoride in water (A) and methanol (B). The gradient elution both in ESI positive and negative mode was 5% B from 0 to 3 min and then increased for B as follows: 5–60% B from 3 to 4 min, followed by isocratic conditions at 60% B until 14 min, and 60-98% B from 14.1 to 17 min. The last step was a decrease of B 98%-5% from 17.1 to 20 min to re-equilibrate with the initial mobile phase composition before the next injection. The flow rate was kept constant at 0.4 ml/min and the column temperature was set at 40°C (see Lopardo et al., 2017). Method validation data are shown in Tables S4.3 and S4.4.

4.1.6 Biomarkers identification

Data extracted from the system after analysis of *in vitro* test samples were processed with MetID software from Advanced Chemistry Development, Inc. (ACD/Labs) for *in silico* prediction of suspected metabolite structures. The exact theoretical accurate mass of the structures was then associated to accurate masses found in the sample

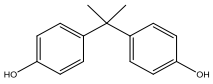
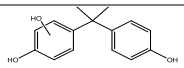
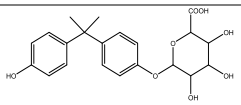
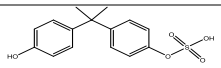
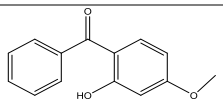
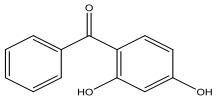
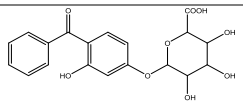
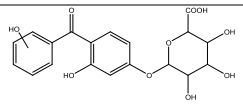
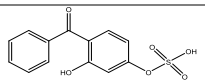
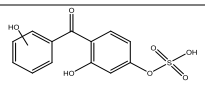
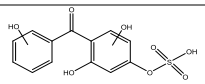
leading in case of a positive match to the identification of a chromatographic peak. The criteria for the elimination of false positives and the identification of actual metabolites amongst the numerous structures suggested were: (i) high mass accuracy (mass error below 5ppm for metabolites produced by *in vitro* studies and below 10 ppm for wastewater analysis) and (ii) lower K_{ow} compared to the non-metabolised compound. The chemical structure was then confirmed through the analysis of the fragmentation pattern (identification of fragments predicted by ACD/Labs MS fragmenter) and isotope pattern (when distinctive). The same workflow was then applied to confirm the presence in urine and wastewater of positively identified metabolites. For details see (Lopardo et al. 2017).

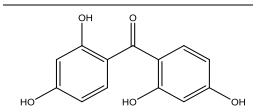
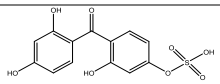
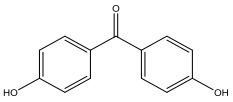
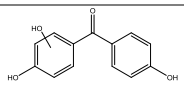
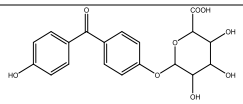
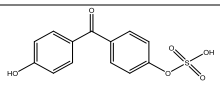
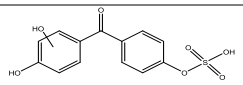
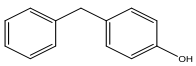
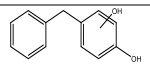
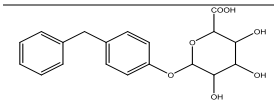
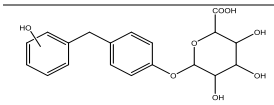
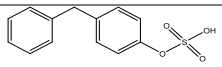
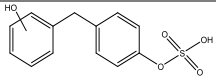
4.5 Results and discussion

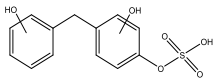
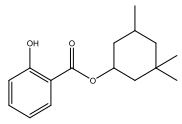
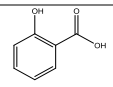
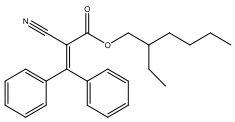
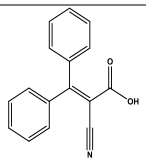
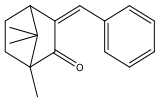
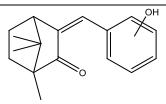
4.1.7 In vitro studies

A comprehensive listing of all observed metabolites, their masses, retention times and metabolite's respective identifying fragments generated at high energy (bbCID) mode is shown in Table S4.2. In most cases, at least one metabolite per UV filter was observed in *in vitro* studies (Table 4.1). Both negative and positive ionisation modes were tested for the analysis, however all the potential metabolites resulted in better ion intensity in the negative ionization mode. The most common enzymatic biotransformation reactions observed for the UV filters investigated were hydroxylation, sulphate conjugation, glucuronidate conjugation and combinations of hydroxylation and phase II biotransformation. The XIC of BP-1 Met2 (m/z 405.0827) produced two chromatographic peaks due to single hydroxylation occurring on two different positions, which lead to the identification of two different metabolites (Fig. S4.1, left). The XIC of 4-BenzPh Met5 (m/z 279.0333) also produced two chromatographic peaks due to single hydroxylation occurring on two different positions, which lead to the identification of two different metabolites (Fig. S4.1, right). Hydrolysis was observed only in the case of HO and OC that were broken down to smaller molecules. All the metabolites produced have not been previously documented in literature.

Table 4.1 Presence of the metabolites identified via in vitro studies in the analysed matrices

Compound	Metabolites identified via *in vitro* studies		Matrices					
			Urine	June WW	July WW	August WW	Sept WW	October WW
 **BPA**	Parent	✓*	< LOD	✓	✓	✓	✓	✓
	 Parent + O (BPA Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + $C_6H_8O_6$ (BPA Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O_3S (BPA Met3)	✓	< LOD	✓	< LOD	✓	✓	✓
 **BP-3**	Parent(Ye et al. 2005)		✓	✓	✓	✓	✓	✓
	BP-1(Gonzalez et al. 2008; León et al. 2010)		✓	✓	✓	✓	✓	✓
 **BP-1**	Parent	✓*	✓	✓	✓	✓	✓	✓
	 Parent + $C_6H_8O_6$ (BP-1 Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + $C_6H_8O_6$ (BP-1 Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O_3S (BP-1 Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + O_3S (BP-1 Met4)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + O + O_3S **** (BP-1 Met5)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

 **BP-2**	 Parent + O_3S **** (BP-2 Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
 **4,4-DHBP**	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O (4,4-DHBP Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + $C_6H_8O_6$ (4,4-DHBP Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O_3S (4,4-DHBP Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + O_3S (4,4-DHBP Me4)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
 **4-BenzPh**	Parent	✓*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O (4-BenzPh Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + $C_6H_8O_6$ (4-BenzPh Met2)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O + $C_6H_8O_6$ (4-BenzPh Met3)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	 Parent + O_3S (4-BenzPh Met4)	✓	< LOD	< LOD	< LOD	✓	< LOD	< LOD
	 Parent + O + O_3S (4-BenzPh Met5)	✓	< LOD	✓	✓	✓	< LOD	✓

	 Parent + O +O + O_3S (4-BenzPh Met6)	✓	✓	< LOD	< LOD	< LOD	< LOD	< LOD
 **HO**	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	 Parent - C_9H_{16} (HO Met1)	✓	< LOD	✓	✓	✓	✓	✓
 **OC**	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	 Parent – C_8H_{16} (OC Met1)	✓	✓	✓	✓	✓	✓	✓
 **3-BC**	Parent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	 Parent + O (3-BC Met1)	✓	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

***present as non-metabolized residue**

**** n.d. (non detectable)**

***** WW (wastewater)**

******same accurate mass and retention time**

4.1.8 **In vivo pooled urine assay**

The *in vivo* pooled urine assay led to identification of two metabolites that were also identified via *in vitro* studies: 4-BenzPh Met6 and OC Met1 (Table 4.1). The identification of the 4-BenzPh Met6 and OC Met1 in urine is reported in Figure 4.1. Figure 4.1a shows the XIC of 4-BenzPh twice hydroxylated and sulphated (m/z 295.0279; Fig. 4.1b) with elemental composition of the deprotonated molecule denoting $C_{13}H_{12}O_6S$- (1.0 ppm mass error). The analysis of the high-energy spectrum (bbCID mode; Fig. 4.1c) led to the identification of a fragment (m/z 215.0706) which corresponded with the loss of [SO_3] from the precursor ion. Figure 4.1d shows the XIC of the hydrolytic metabolite of OC (m/z 248.0711; Fig. 4.1e) with elemental composition of the deprotonated molecule denoting $C_{15}H_{11}NO_2$- (2.4 ppm mass error). The analysis of the high-energy spectrum (bbCID mode; Fig. 4.1f) led to the identification of a fragment (m/z 204.0815) which corresponded with the loss of [COO] from the precursor ion. Moreover, BP-3 and BP-1 were also identified in the

analysed urine sample (Fig. S4.2). It is worth mentioning here that BP-1 can be also formed as a metabolite of BP-3. Interestingly, no BPA conjugated metabolites were observed in the analysed pooled urine samples.

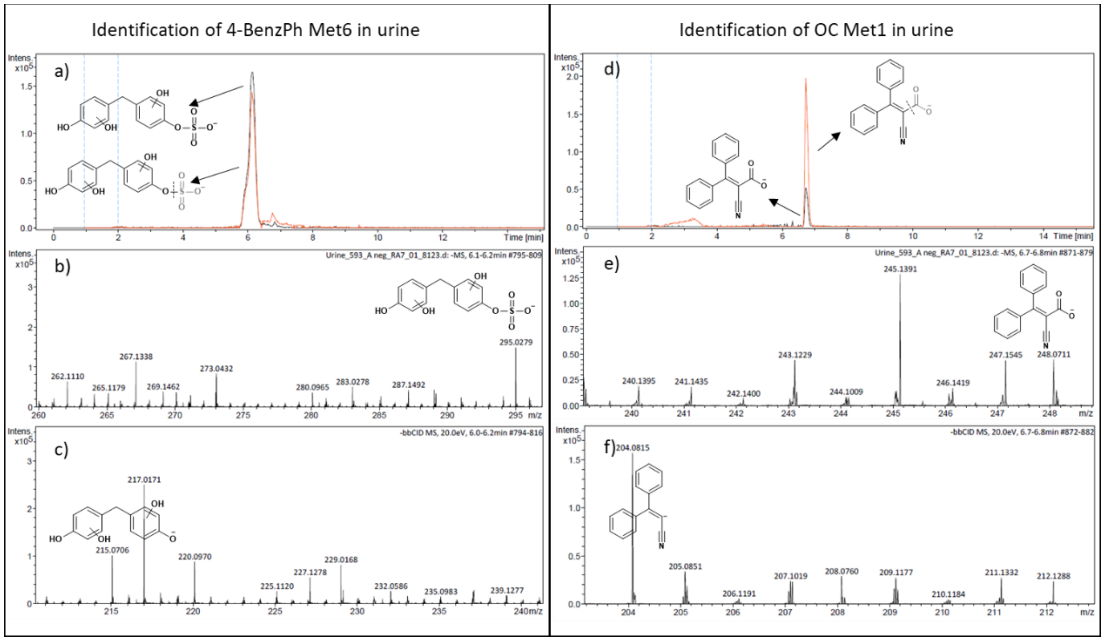


Figure 4.1 Detection and identification of 4-BenzPh Met6 (a;b;c) and OC Met1 (d;e;f) by UHPLC-QTOF-MS following in vivo pooled urine assay. (a) XICs at m/z 295.0282 (0.005-Da mass-window width) and 215.0706. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of 4-BenzPh Met6 and fragment ion observed. (d) XICs at m/z 248.0717 (0.005-Da mass-window width) and 204.0815. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed.

4.1.9 **Wastewater fingerprinting assay**

Finally, the presence of the discovered metabolites was investigated in wastewater to selected biomarkers of public exposure to these chemicals. Analysis of untreated wastewater samples serving five large communities ranging between 17 and 100 thousand people was undertaken. The compounds detected in wastewater are summarised in Table 4.1. Among them are: BPA and BPA-Met2, BP-3, BP-1, HO-Met1, OC-Met1, 4-BenzPh-Met4 and 4-BenzPh-Met5.

Initial analysis of samples, performed using ACDLabs software, identified four potential metabolites: (1) the hydrolytic product of octocrylene (OC Met1), (2) the hydrolytic product of homosalate (HO Met1), (3) 4-benzylphenol sulphated (4-BenzPh-Met4) and (4) 4-benzylphenol sulphated and hydroxylated (4-BenzPh-Met5). The identification of the four metabolites is reported in Figures 4.2 and 3.

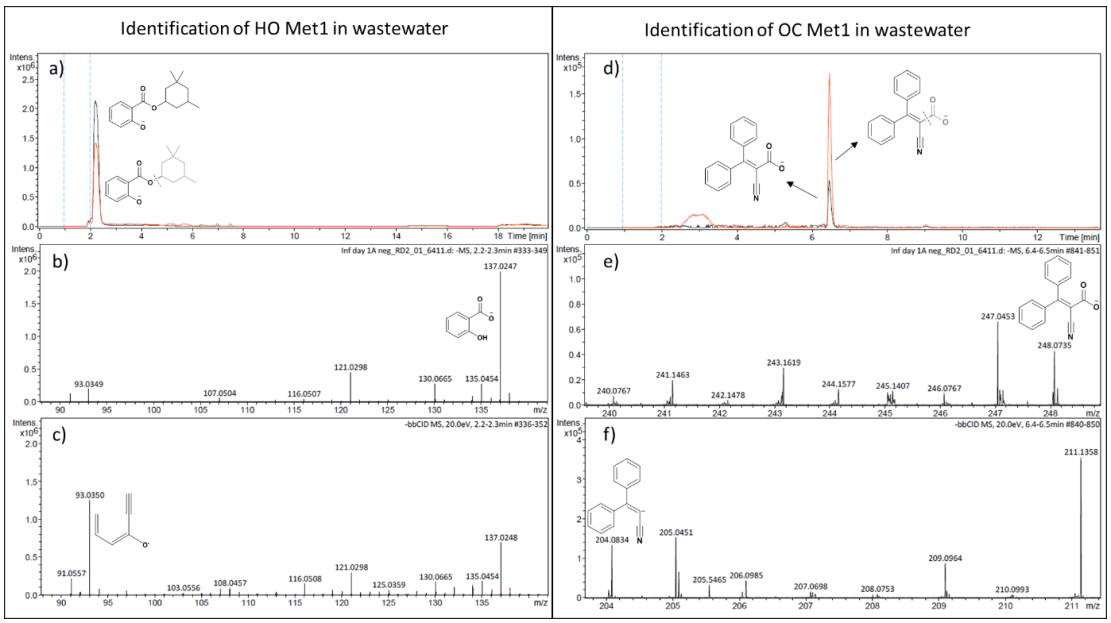


Figure 4.2 Detection and identification of HO Met1 (a;b;c) and OC Met1 (d;e;f) by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 137.0244 (0.005-Da mass-window width) and 93.0350. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of HO Met1 and fragment ion observed. (d) XICs at m/z 248.0717 (0.005-Da mass-window width) and 204.0834. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed.

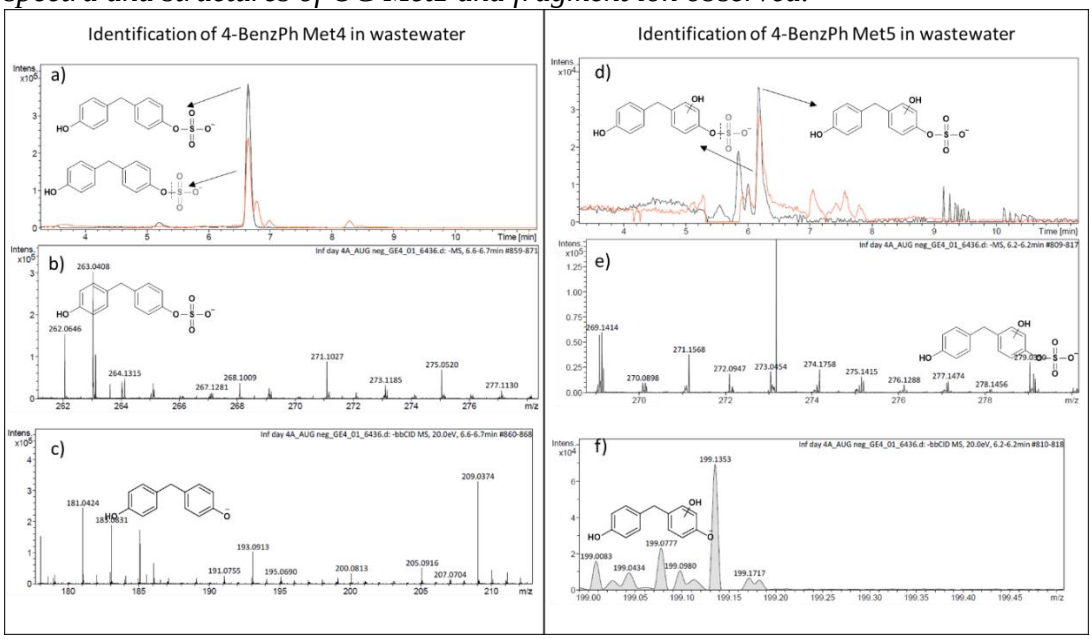


Figure 4.3 Detection and identification of 4-BenzPh Met4 (a;b;c) and 4-BenzPh Met5 (d;e;f) by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 263.0384 (0.005-Da mass-window width) and 183.0815. Low-energy (full-scan analysis) (b) and High-energy (bbCID mode) (c) spectra and structures of HO Met1 and fragment ion observed. (d) XICs at m/z 279.0333 (0.005-Da mass-window width) and 199.0765. Low-energy (full-scan analysis) (e) and High-energy (bbCID mode) (f) spectra and structures of OC Met1 and fragment ion observed

Figure 4.2a shows the XIC of HO Met1 (m/z 137.0247; Fig. 4.2b) with elemental composition of the deprotonated molecule denoting $C_7H_5O_3$- (2.2 ppm mass error). The analysis of the high energy spectrum (bbCID mode; Fig. 4.2c) led to the identification of a fragment (m/z 93.0350) which corresponded with the loss of [C_9H_{18}] from the precursor ion. It is worth mentioning here that HO Met1, salicylic acid, has different application, e.g. as a pharmaceutical. Further work is needed to verify contributions from HO in the overall salicylic acid load in wastewater.

Figure 4.2d shows the XIC of OC Met1 (m/z 248.0735; Fig. 4.2e) with elemental composition of the deprotonated molecule denoting $C_{15}H_{11}NO_2$- (7.2 ppm mass error). The analysis of the high-energy spectrum (bbCID mode, Fig. 4.2f) led to the identification of a fragment (m/z 204.0834) which corresponded with the loss of [COO] from the precursor ion.

Figure 4.3a shows the XIC of 4-BenzPh-Met4 (m/z 263.0408; Fig. 4.3b) with elemental composition of the deprotonated molecule denoting $C_{13}H_{11}O_4S$- (9.1 ppm mass error). The analysis of the high energy spectrum (bbCID mode; Fig. 4.3c) led to the identification of a fragment (m/z 183.0815) which corresponded with the loss of [SO_3] from the precursor ion.

Figure 4.3d shows the XIC of 4-BenzPh-Met5 (m/z 279.0350; Fig. 4.2e) with elemental composition of the deprotonated molecule denoting $C_{13}H_{11}O_5S$ - (6.1 ppm mass error). The analysis of the high-energy spectrum (bbCID mode, Fig. 4.3f) led to the identification of a fragment (m/z 199.0765) which corresponded with the loss of [COO] from the precursor ion.

The relative intensities between the detected ions showed consistency compared to the *in vitro* experiments. BP-3, BP-1 (Fig. S4.3) and sulphated BPA (Fig. 4.4) were also identified in the analysed wastewater samples.

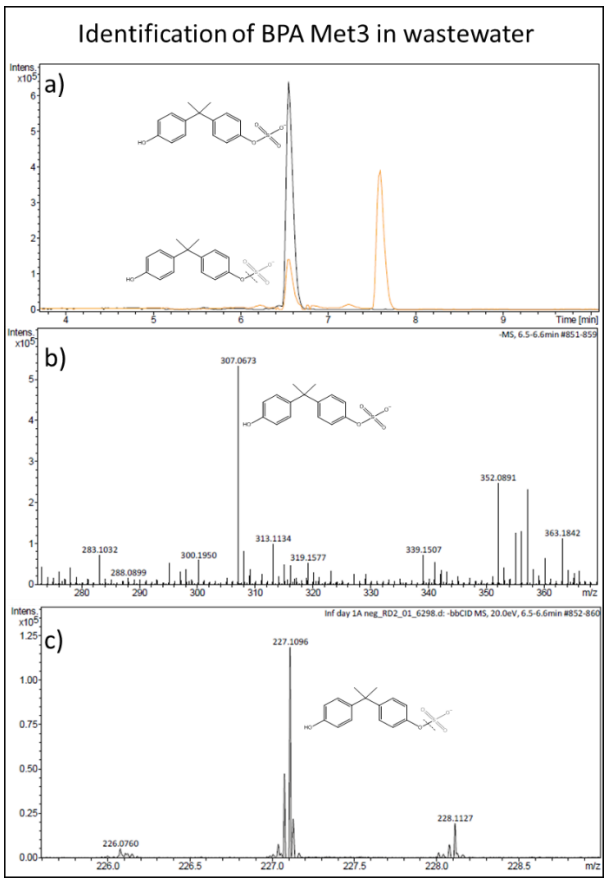


Figure 4.4 Detection and identification of BPA Met3 by UHPLC-QTOF-MS following wastewater fingerprinting assay. (a) XICs at m/z 307.0646 (0.005-Da mass-window width) and 227.1096. (b) Low-energy (full-scan analysis) and (c) High-energy (bbCID mode) spectra and structures of BPA Met 3 and fragment ion observed

The presence of all the above metabolites detected in wastewater (including BPA sulphate) has not been previously documented in literature. As a result, there is no knowledge of the occurrence and effects of these chemicals in the context of environmental and public health. This study has proven that selected chemicals (4-benzylphenol, homosalate, octocrylene) used in personal care and consumer products undergo metabolism in humans leading to the formation of new chemicals that are found in communal wastewater and can be discharged into the receiving environment. Further work is required (a) to verify public exposure to EDCs utilising WBE and (b) to understand fate and biological effects of EDCs and their metabolites in exposed ecosystems.

To summarise, out of eleven chemicals were selected in this study, only two (BP-1, BP-3) were identified in urine and three (BPA, BP1, BP3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-

BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in urine and/or wastewater. Such difference in detection patterns of selected EDCs and their metabolites was expected as:

(1) They have different applications (Table S4.5), i.e. some (e.g. BP-3, HO, OC) are used mainly in personal care products and are applied directly to skin. Hence, due to direct exposure, they are more likely to be excreted with urine and discharged with wastewater as a result of e.g. showering.
(2) They differ in frequency and volume of usage, i.e. only homosalate, octocrylene, and benzophenone-3 are in the top 10 of the most commonly used UV filters in the UK (Kerr 2011). Their usage is season dependent.
(3) Some could not be detected due to analytical constraints, e.g. HO, OC and 3-BC have higher hydrophobicity and could be more amenable to GC analysis.
(4) Pooled urine and wastewater samples were collected at different time and location, therefore representing populations with different exposure signature.

The presence of BPA, OC and HO and 4-benzylphenol metabolic residues as well as the confirmation of benzophenones in urine and wastewater is of significant importance. For example, BPA and 4-BenzPh are mostly employed in the production of epoxy-phenolic resins and polycarbonate plastics used as thin protecting coatings on the interior surface of metal cans but it can be also found in many products like dental sealants, food packaging, beverage cans, personal care products, baby bottles, building materials and flame-retardant materials. Consequently, there is a widespread potential for human exposure, which this study reports. Epidemiological studies along with animal and *in vitro* experiments reported BPA exposure as a potential cause of several adverse health effects, such as cancer, obesity and disorders in endocrine, renal and reproductive systems (Joint Fao Oms Expert Committee On Food Additives 2010). Benzophenones, 4-BenzPh, OC and HO have been demonstrated in many studies to possess endocrine disrupting properties at rather high concentrations (Akahori et al. 2008; Fent et al. 2008; Kunz and Fent 2006b; Schlumpf et al. 2001), but as highlighted by Kunz and Fent (Kunz and Fent 2006a), for an adequate risk assessment when investigating endocrine disrupting properties, it becomes crucial to consider exposure to compound mixtures rather than single compounds given the possibility for a synergistic effects. Also, given their lipophilic nature, these

compounds show great potential for bioaccumulation (Gago-Ferrero et al. 2015) and for ecotoxicological effects in aquatic ecosystems (Díaz-Cruz and Barceló 2009; Fent et al. 2008; Kaiser et al. 2012).

4.6 Conclusions

This study presents a comprehensive examination of the *in vitro* metabolism of eleven EDCs used in personal care and consumer products (Table S4.5) in order to identify suitable biomarkers of exposure and their analysis in biological matrices such us urine and wastewater, and ultimately to verify the extent of public internal and external exposure to these chemicals. External exposure indicates the whole EDC dose to which an organism was exposed. Internal exposure indicates only the fraction of the initial chemical dose that was absorbed by and distributed throughout the body. The biotrasformations observed were mainly phase I processes such as hydrolysis and hydroxylation together with phase II conjugation reactions such as sulphation and glucuronidation. Eleven chemicals were selected in this study. Only two (BP-1, BP-3) were identified in urine and three (BPA, BP1, BP3) in wastewater. Five newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were detected in urine and/or wastewater.

This new approach towards biomarkers selection shows a significant potential, especially in its future application in verification of public exposure to chemicals using WBE. Furthermore, an opportunity for further studies focussing on understanding of fate and effects of EDCs and their metabolites in the aquatic environment needs to be emphasised too. It is apparent that further work is needed to increase the pool of available biomarkers, increase selectivity and sensitivity of analytical methods, and analyse larger sets of samples allowing for the verification of spatial and temporal changes in exposure patterns to chemicals. When combined with further studies in this area, researchers will have a basis for fine tuning *in vitro* assays to better produce results useful for comprehensive metabolic profiling of both known and novel chemicals.

4.7 Acknowledgments

The support of the Leverhulme Trust (Project No RPG-2013-297) is greatly appreciated. We would also like to acknowledge Trevor Shine at TICTAC Communications (St George's University of London, United Kingdom) for provision of pooled urine samples. All data supporting this study are provided as supporting information accompanying this paper.

4.8 Supplementary material

4.1.10 List of tables

4.1.11 List of figures

4.1.12 List of reports (appendix B)

(Available online)

Table S4.1 List of compounds selected and their physical-chemical properties (https://scifinder.cas.org)

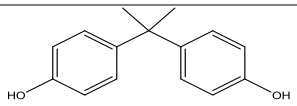
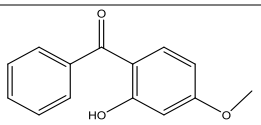
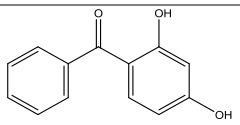
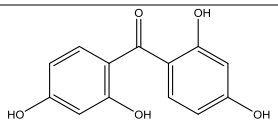
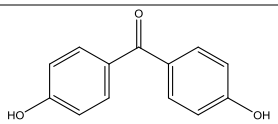
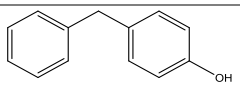
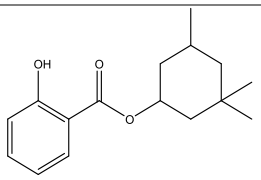
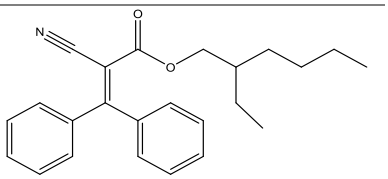
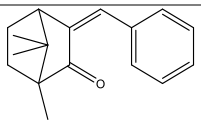
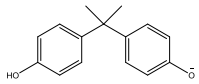
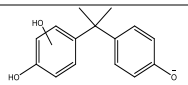
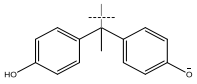
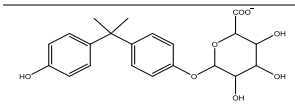
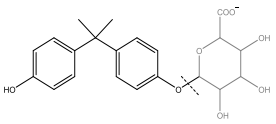
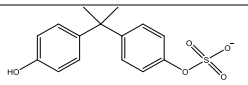
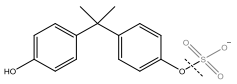
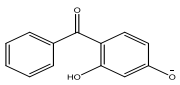
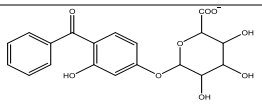
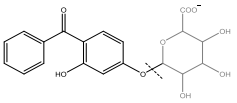
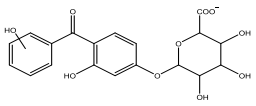
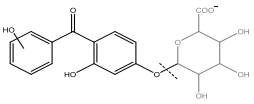
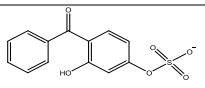
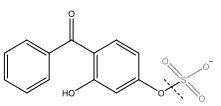
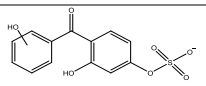
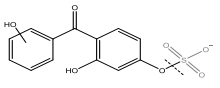
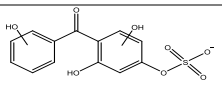
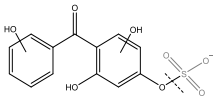
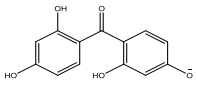
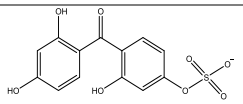
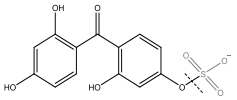
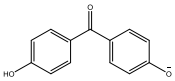
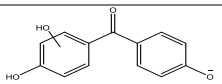
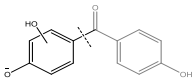
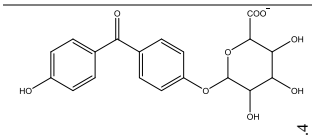
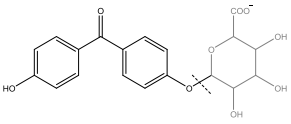
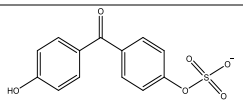
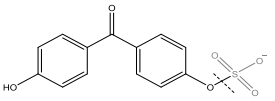
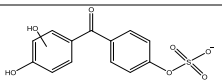
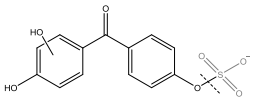
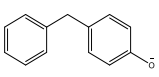
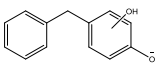
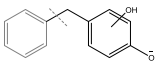
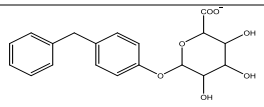
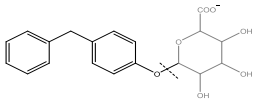
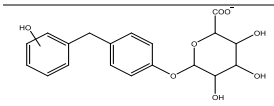
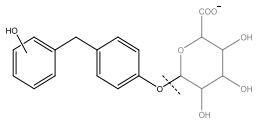
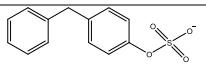
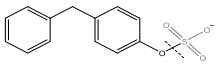
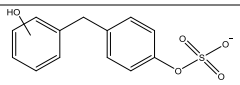
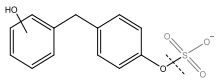
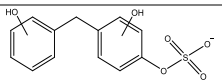
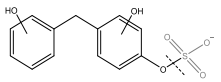
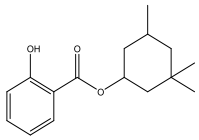
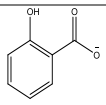
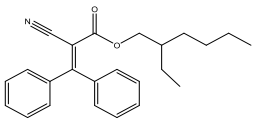
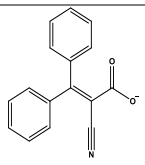
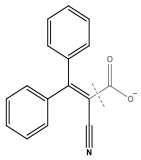
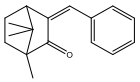
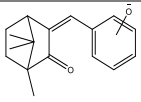
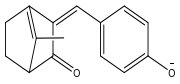
Chemical (Abbreviation)	CAS number	pKa	Log P	Molecular Weight	Formula	Structure
Bisphenol A (BPA)	80-05-7	10.29	3.43	228.3	$C_{15}H_{16}O_2$	
Benzophenone 3 (BP3)	131-57-7	8.03	3.64	228.2	$C_{14}H_{12}O_3$	
Benzophenone-1 (BP1)	131-56-6	7.72	3.15	214.22	$C_{13}H_{10}O_3$	
Benzophenone-2 (BP2)	131-55-5	6.98	3.09	246.22	$C_{13}H_{10}O_5$	
4,4'-Dihydroxybenzophenone (4,4'-DHBP)	611-99-4	7.67	2.63	214.22	$C_{13}H_{10}O_3$	
4-benzylphenol (4-BenzPh)	101-53-1	10.23	3.47	184.2	$C_{13}H_{12}O$	
Homosalate (HO)	118-56-9	8.1	5.82	262.3	$C_{16}H_{22}O_3$	
Octocrylene (OC)	6197-30-4		7.53	361.5	$C_{24}H_{27}NO_2$	
3-Benzylidene Camphor (3-BC)	15087-24-8		2.84	240.3	$C_{17}H_{20}O$	

Table S4.2 List of metabolites produced via in vitro studies, suggested structures and relative abundances. Accurate masses, retention times and confirmation fragmets structures produced in MS/MS mode are also provided.

Compound				Metabolites					
	Ret. time(min)	m/z	Mass error (ppm)	Suggested structure Type of biotransformation (abbreviation)	Met relative abundance (%)	Ret. time (min)	m/z	Average mass error (Δppm)	Fragment structures
BPA 	7.7	227.1078	1.8	 Parent + O (BPA Met1)	1	7.2	243.1025	0.8	
				 Parent + $C_6H_8O_6$ (BPA Met2)	90	6.6	403.1398	3.8	
				 Parent + O_3S (BPA Met3)	9	6.85	307.0646	1.3	
BP-1 	7.8	213.0557	3.3	 Parent + $C_6H_8O_6$ (BP-1 Met1)	55	6.5	389.0878	2.6	
				 Parent + O + $C_6H_8O_6$ (BP-1 Met2)	4.2 and 6.3	6.4 and 6.6	405.0827	1	
				 Parent + O_3S (BP-1 Met3)	1.5	6.8	293.0125	0.0	

				 Parent + O + O_3S (BP-1 Met4)	25.2	6.7	309.0074	0.6	
				 Parent + O + O + O_3S (BP-1 Met5)	7.8	6.2	325.0024	0.3	
BP-2 	6.8	245.0455	3.2	 Parent + O_3S (BP-2 Met1)	100	6.25	325.0025	3.3	
4,4-DHBP 	6.7	213.0557	3.8	 Parent + O (4,4-DHBP Met1)	58.8	6.5	229.0506	2.6	
				 Parent + $C_6H_8O_6$ (4,4-DHBP Met2)	1.4	5.1	389.0878	0.5	
				 Parent + O_3S (4,4-DHBP Met3)	22.6	6.25	293.0125	2.1	
				 Parent + O + O_3S (4,4-DHBP Me4)	17.2	6.15	309.0074	1.0	
4-BenzPh 	8.3	183.0815	4.1	 Parent + O (4-BenzPh Met1)	26.9	7.6	199.0765	3.5	

			 Parent + $C_6H_8O_6$ (4-BenzPh Met2)	22	6.8	359.1136	3.1	
			 Parent + O + $C_6H_8O_6$ (4-BenzPh Met3)	1	6.7	375.1085	4.8	
			 Parent + O_3S (4-BenzPh Met4)	21.8	7.0	263.0384	5.3	
			 Parent + O + O_3S (4-BenzPh Met5)	6 and 19	6.5 and 6.9	279.0333	4.9	
			 Parent + O +O + O_3S (4-BenzPh Met6)	3.4	6.4	295.0282	4.9	
HO		n.d.	n.d.	n.d.	 Parent - C_9H_{16} (HO Met1)	100	2.9	137.0244	4.0	
OC		n.d.	n.d.	n.d.	 Parent – C_8H_{16} (OC Met1)	100	6.8	248.0717	4.9	
3-BC		n.d.	n.d.	n.d.	 Parent + O (3-BC Met1)	100	8.9	255.1391	2.8	

n.d. non-detectable

Table S 4.3 UHPLC-QTOF instrument performance parameters

Analyte	IS	Rt [min]	Linearity		Intra-day instrument performance		Inter-day instrument performance		IDL [ng L^{-1}]	IQL [ng L^{-1}]
					Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)		
BPA	Bisphenol A-d16	7.7	0.28-28	0.9991	114.8	2.5	114.8	9.4	1.4	4.7
BP-3	Benzophenone-3-d5	9.2	0.07-54	0.9992	104.4	8.4	113.5	4.2	132.44	441.481
BP-1	4-Cl-3-methylphenol-d2	7.8	0.13-31	0.9975	110.4	8.3	110.4	15.0	1.5	5.0
BP-2	Bezafibrate-d6	6.8	0.1-88	0.9996	98.0	6.5	98.0	10.1	1.1	3.6
4.4-DHBP	Bezafibrate-d6	6.6	0.09-21.5	0.9994	109.2	11.4	95.2	7.8	1.5	5.1
4-BenzPh	Bezafibrate-d6	8.3	0.1-55	0.9995	138.8	9.8	136.6	26.5	5.7	19.0

Table S4.4 SPE-UHPLC-QTOF method performance parameters

Analyte	Wastewater influent		
	SPE recover [%]	MDL [ng L^{-1}]	MQL [ng L^{-1}]
BPA	100.4	0.004	0.012
BP-3	86.1	0.285	0.950
BP-1	89.7	0.003	0.011
BP-2	102.9	0.003	0.009
4,4-DHBP	106.6	0.004	0.013
4-BenzPh	105.2	0.015	0.050

Table S4.5 Purpose, commercial use and potential exposure sources of the selected compounds

Compound	Purpose	Main commercial use/source	Reference
Bisphenol A (BPA)	Byproduct/Intermediate/Reactant, Plastic/Rubber	Protective linings of canned food container, food packaging, dental sealants; thermal receipts and paper currencies	European Food Safety Authority (EFSA); (Biedermann et al. 2010); https://endocrinedisruption.org/
Benzophenone 3 (BP3)	UV filter	Personal care products including sunscreen, cosmetics, lotions, fragrances, shampoos, body washes, soap, and insect repellents	US Dept of Health & Human Services. Household Products Database; Official Journal of European union; https://endocrinedisruption.org/
Benzophenone-1 (BP1)	UV filter	Food additive, Household product ingredient, Metabolite/Degradate, Personal care product/Cosmetic ingredient, Pesticide ingredient, Plastic/Rubber	US Dept of Health & Human Services. Household Products Database; European commission; https://endocrinedisruption.org/

Benzophenone-2 (BP2)	UV filter	Food additive, Household product ingredient, Personal care product/Cosmetic ingredient, Pesticide ingredient, Plastic/Rubber	US Dept of Health & Human Services. Household Products Database; European commission; https://endocrinedisruption.org/
4,4'-Dihydroxybenzophenone (4,4-DHBP)	UV filter	Industrial additive, Personal care product/Cosmetic ingredient, Plastic/Rubber	https://endocrinedisruption.org/
4-benzylphenol (4-BenzPh)	Byproduct/Intermediate/Reactant, Plastic/Rubber; Antiseptic	Medical/Veterinary/Research, Plastic/Rubber	https://endocrinedisruption.org/ (Hashimoto et al. 2001)
Homosalate (HO)	UV filter	Personal care product/Cosmetic ingredient/ Pesticide ingredient	US Dept of Health & Human Services. Household Products Database; European commission; https://endocrinedisruption.org/
Octocrylene (OC)	UV filter	Personal care product/Cosmetic ingredient	European commission; https://endocrinedisruption.org/
3-Benzylidene Camphor (3-BC)	UV filter	Household product ingredient, Medical/Veterinary/Research, Personal care product/Cosmetic ingredient	https://endocrinedisruption.org/

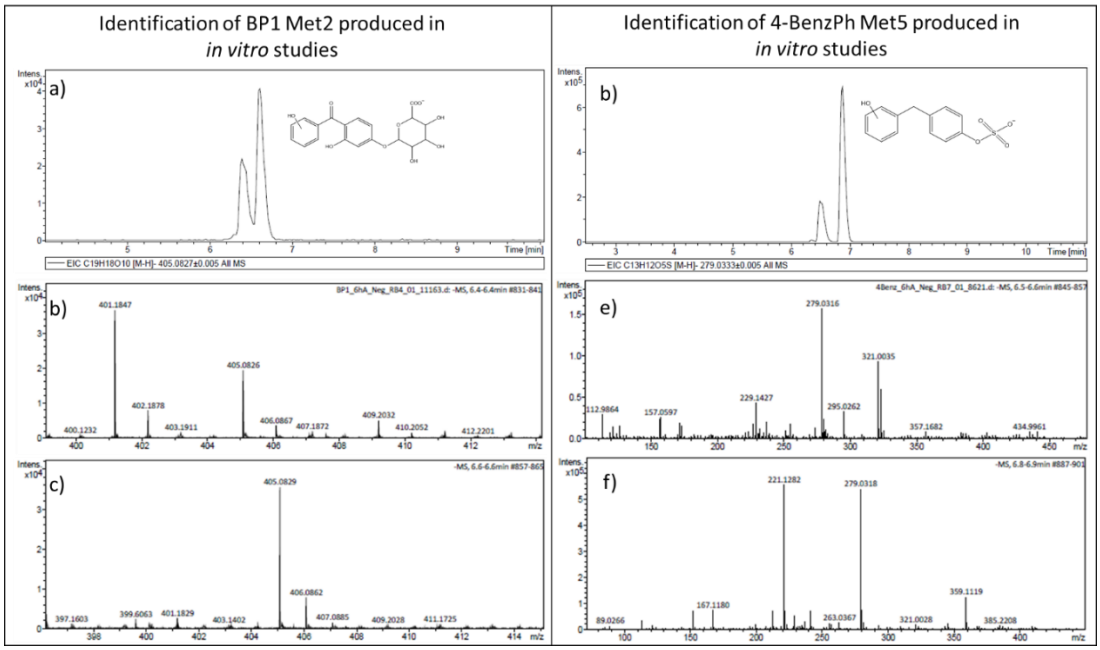


Figure S4.1 XIC at m/z 405.0827 (0.005-Da mass-window width) (a) and mass spectra of the peak eluted at 6.4 minutes (b) and of the peak eluted at 6.6 minutes (c). XIC at m/z 279.0333 (0.005-Da mass-window width) (d) and mass spectra of the peak eluted at 6.5 minutes (b) and of the peak eluted at 6.9 minutes (c).

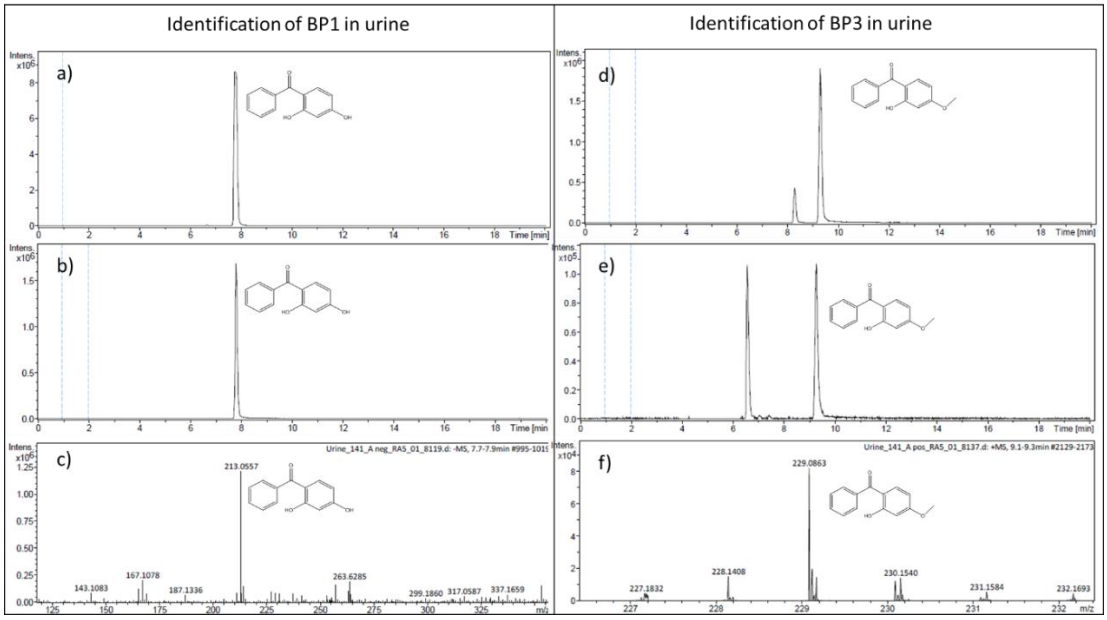


Figure S4.2 Detection and identification of BP-1 (m/z 213.0557) and BP-3 (229.0859) by UHPLC-QTOF-MS following analysis of standard solutions and in vivo pooled urine assay. (a) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a BP-1 standard solution; (b) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a pooled urine sample and mass spectra (c). (d) XIC at m/z 229.0859 (0.005-Da mass-window width) of a BP-3 standard solution; (e) XIC at and m/z 229.0859 (0.005-Da mass-window width) of a pooled urine sample and mass spectra (f).

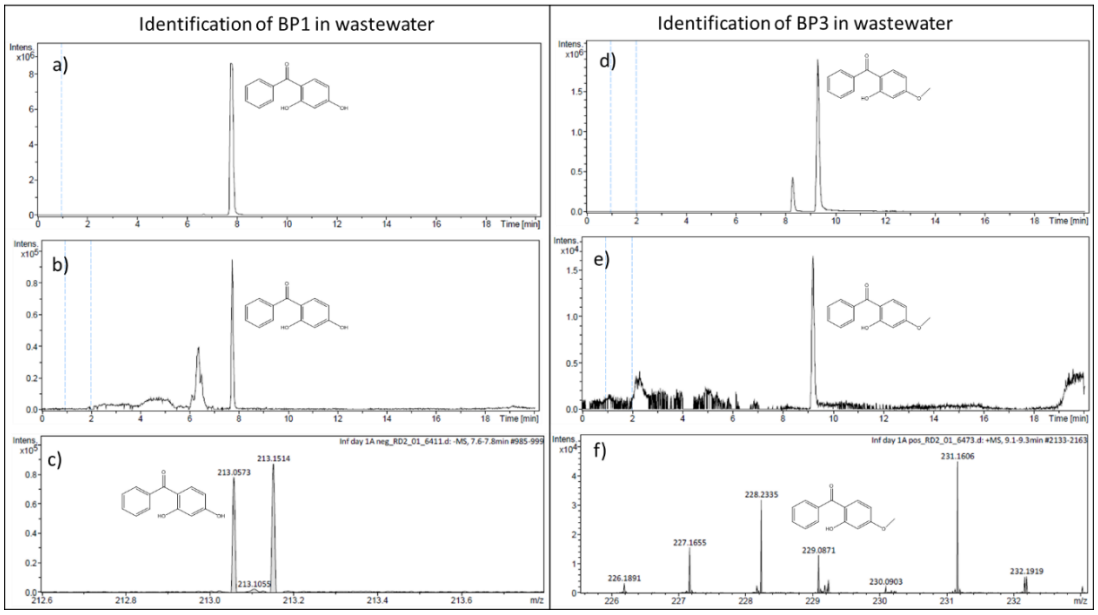


Figure S4.3 Detection and identification of BP-1 (m/z 213.0557) and BP-3 (229.0859) by UHPLC-QTOF-MS following analysis of standard solutions and wastewater

fingerprinting assay. (a) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a BP-1 standard solution; (b) XIC at and m/z 213.0557 (0.005-Da mass-window width) of a wastewater sample and mass spectra (c). (d) XIC at m/z 229.0859 (0.005-Da mass-window width) of a BP-3 standard solution; (e) XIC at and m/z 229.0859 (0.005-Da mass-window width) of a wastewater sample and mass spectra (f).

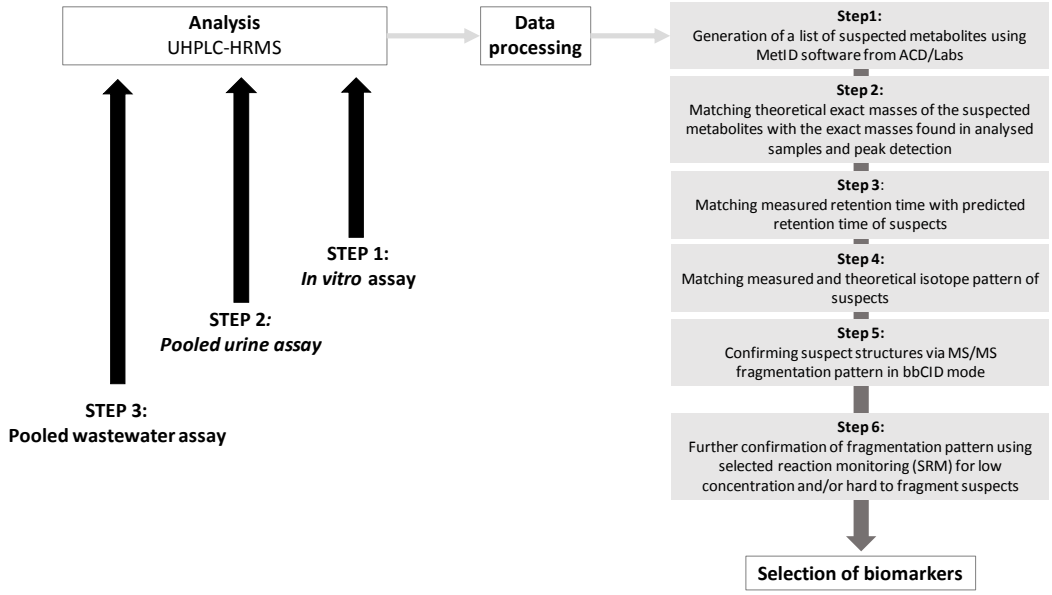


Figure S4.4 A systematic workflow for identification and selection of human biomarkers of exposure to environmental contaminants via combined in vitro, pooled urine and pooled wastewater profiling assay

4.9 References

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5 A new analytical framework for multi-residue analysis of chemically diverse endocrine disruptors in complex environmental matrices utilising ultra-performance liquid chromatography coupled with high resolution tandem quadrupole time-of-flight mass spectrometry

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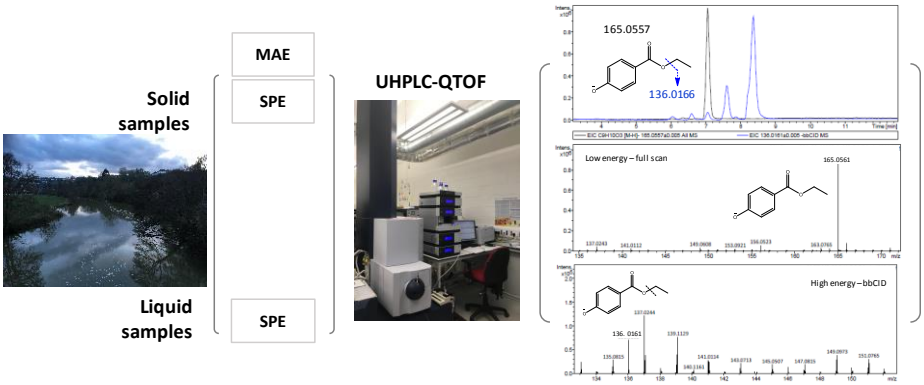
5.1 Graphical abstract



Keywords Endocrine disruptor, personal care products, wastewater, sludge, environment, high resolution LC-MS

5.2 Abstract

The scope of this work is to report a new analytical methodology to assess the presence of 37 chemically diverse endocrine disrupting chemicals (EDCs) used in personal care and consumer products in diverse environmental matrices (both solid and liquid matrices) utilising liquid chromatography coupled with tandem quadrupole time-of-flight mass spectrometry (UHPLC-ESI-MS/MS). Solid-phase extraction (SPE) was applied to liquid matrices in order to reduce matrix effects, provide required sample concentration and ultimately, high sensitivity and selectivity of measurements. SPE recoveries in liquid samples ranged from 49 to 140% with method quantification limits not exceeding 1 ng L^{-1} for the majority of EDCs. Microwave assisted extraction (MAE) was applied to solid samples and when followed by SPE, it permitted the analysis of 22 EDCs in digested sludge. MAE/SPE recoveries varied from 11 to 186 % and MQLs between 0.03 and 8.1 ng g^{-1} with the majority of compounds showing MQLs below 2 ng g^{-1}. Mass error for quantifier and qualifier ions was below 5 ppm when analysing river water and effluent wastewater and below 10 ppm when analysing influent wastewater and solid samples. The method was successfully applied to environmental samples, with 33 EDCs identified and quantified in wastewater and receiving waters. In addition, several of them were found in digested sludge, which confirms that for a more comprehensive understanding of exposure patterns and environmental impact, analysis of solids cannot be neglected. Finally post acquisition data mining permitted the identification and quantification for a metabolite of BPA and the identification of a metabolite of 4-Cl-3-methylphenol.

5.3 Introduction

Many chemicals in personal care and consumer products such as preservatives, UV filters, plasticizers, fragrances, antimicrobials, pesticides and flame retardants are suspected to have or are recognised as endocrine system disruptors (Tijani et al. 2016; Wong and Durrani 2017). Unfortunately, there is lack or limited understanding of the extent and patterns of human exposure to these chemicals. This is despite a critical need to obtain such data at a population level to inform future regulations.

Several papers have been published attributing serious health issues to different EDCs and calling for more research and regulations (Kovacic 2010; McCormick et al. 2010; Testai et al. 2013; Vandenberg et al. 2012) but there are also some that argue that the problem is quite small when compared to natural hormone affecting compounds that are consumed with food, and that therefore think that such regulations would be unnecessary (Lamb et al. 2014; Nohynek et al. 2013; Rhomberg and Goodman 2012). Therefore, accurate and reliable exposure assessments of EDCs are required if any more regulatory measures are to be taken, but it is difficult to do such an assessment at a population level. During the past decade the field of wastewater based epidemiology (WBE) has been developed to address the need for accurate and reliable assessment of population based exposure to e.g. illegal drugs (Daughton 2001; Zuccato et al. 2005). WBE relies on the analysis of target compounds and their metabolites in wastewater so that accurate exposure levels can be back-calculated and it has been proven to give usage estimates of illegal drugs that match official numbers. Using a WBE approach it is also possible to follow the exposure trends on a real time basis so that trends over weeks or shorter and longer time periods can be observed, e.g. the increased use of some illegal drugs on weekends(Zuccato et al. 2008b).

Several studies have investigated the presence of EDCs in different types of samples including surface waters, wastewater, digested sludge and solid samples (Azzouz and Ballesteros 2014, 2016; Benigni et al. 2015; Camilleri et al. 2014; Carvalho et al. 2015; Gorga et al. 2013; Marti and Batista 2014; Petrie et al. 2015; Tan et al. 2008; Zhang et al. 2011). These studies have utilized different analytical techniques for both sample preparation, separation and detection. The most common method for sample preparation is solid phase extraction (SPE), which has been used both online (Camilleri et al. 2014) and offline (Azzouz and Ballesteros 2014; Carvalho et al. 2015;

Gorga et al. 2013), but there are examples where stir bar sorptive extraction (SBSE) has been used instead (Tan et al. 2008). A few of the studies have analysed their target analytes using gas chromatography (GC) hyphened to mass spectrometer (MS) (Azzouz and Ballesteros 2014). Others have used liquid chromatography (LC) coupled with MS (Camilleri et al. 2014; Carvalho et al. 2015; Gorga et al. 2013). Sosa-Ferrera et al. (Sosa-Ferrera et al. 2013) compiled and compared different LC based methods that have been used to analyse different EDCs. However, most of these studies focus on a small number of EDCs generally from a limited number of chemical classes (Gago-Ferrero et al. 2013; He et al. 2017; Santiago-Morales et al. 2012; Wu and Ding 2010) and/or are often investigated alongside other pharmaceuticals (Azzouz and Ballesteros 2016; Chen et al. 2010; Gasperi et al. 2014; Gorga et al. 2013; Petre et al. 2016; Zhang et al. 2011). Moreover, they mostly use targeted MS detection meaning that their results cannot be used for retrospective data analysis.

The aim of this work was to develop a robust analytical framework for selective and sensitive multi-residue analysis of structurally diverse EDCs (ranging from fragrances to brominated flame retardants) in wastewater (both solid and liquid samples) and receiving environment while giving the capability to undertake retrospective data analysis on analysed samples. To achieve this, ultra-high performance liquid chromatography (UHPLC) hyphenated to high resolution mass spectrometry (HRMS) was used.

5.4 Materials and methods

5.4.1 Chemicals

The following analytes were targeted in this study (Table S5.1): 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2-ethylhexanoic acid, 2-naphthol, 4,4'-dihydroxybenzophenone, 4-benzylphenol, 4-chloro-3,5,dimethylphenol, 4-chloro-3-methylphenol, 4-n-nonylphenol, 4-n-octylphenol, atrazine, benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), benzophenone-4 (BP-4), benzylparaben, bisphenol A (BPA), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE-2-Cl), butylparaben, chlorothymol, dibutyl phthalate (DBP), ethylparaben, galaxolide, 1,2,5,6,9,10-hexabromo-cyclododecane (HBCD), mono(2-ethylhexyl)phthalate (MEHP), methylparaben, monobutyl phthalate (MBP), musk ketone, padimate O, perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), phenylbenzimidazolesulfonic acid (PBSA, ensulizole), prochloraz, propylparaben, tetrabromobisphenol A (TBBPA), triclocarban, triclosan and vinclozolin (see Table S5.1 in the supplementary material section for further details). The internal standards used were: 4-chloro-3-methylphenol-d_2, atrazine-d_5, bezafibrate-d_6, BP-3-d_5, triclosan d_3 and triclocarban-d_4 (QMX (UK) or TRC (UK). Water was purified using a Milli-Q purification system from Millipore (Nottingham, UK). Methanol, formic acid (>95 %), HCl (concentrated), 1M NaOH, 1M NH_4OH, NH_4F and 2-propanol were purchased from Sigma (UK) and Fisher (UK). All solvents used were of LC grade or higher.

Glassware was deactivated using 5 % dimethyldichlorosilane in toluene (DMDCS; Sigma, UK) to prevent losses from analyte adsorption. The deactivation procedure consisted of washing the glassware once with 5 % DMDCS followed by two washes with toluene and lastly three washes with methanol.

5.4.2 Sample collection

Pooled influent and effluent wastewater samples were collected at a wastewater treatment plant using ISCO 3700 portable samplers (RS Hydro, Worcestershire, UK) that were set up to do volume proportional collections of 10 mL portions with an average sampling rate of 15 min. The samples were kept at 4 °C until collection and transported on ice to the laboratory. After spiking with internal standards, pooled 24-h samples were then stored at -18 °C until sample preparation and analysis. Grab

samples were also collected from receiving river waters from upstream and downstream of the effluent discharge point on each sampling day. Digested sludge was collected from an anaerobic digestion plant.

5.4.3 Sample preparation

Liquid matrix - solid-phase extraction. All liquid environmental samples were filtered using GF/F (0.7 µm) filters (Whatman, UK). After filtration samples underwent solid-phase extraction according to below procedures.

100 mL of each sample were transferred to 125 mL plastic bottles (HDPE) and spiked with 25 µL of an internal standard mixture (100 µg L^{-1}). After spiking the samples were extracted using three SPE protocols developed for HLB, MCX and MAX sorbents respectively.

HLB extraction protocol included conditioning of 60 mg 3cc HLB cartridges (Waters, UK) with 2 mL of methanol followed by 2 mL of water. Samples were adjusted to a neutral pH with formic acid (>95 %) or 1 M NaOH and then applied to HLB cartridges using vacuum. After a 30 minute drying step all cartridges were stored in a freezer at -18 °C until elution. Elution was undertaken using 4 mL of methanol.

MCX extraction protocol included conditioning of 60 mg 3cc MCX cartridges (Waters, UK) with 2 mL methanol followed by 2 mL of water with 2 % of formic acid. Samples were adjusted to a pH < 2.5 with the addition of 0.5 mL HCl and then applied using vacuum. After a 30 minute drying step all cartridges were stored in a freezer at -18 °C until elution. Elution was undertaken using 2 mL of methanol followed by 2 mL of methanol with 5 % ammonium hydroxide.

MAX extraction protocol included conditioning of 60 mg 3cc MAX cartridges (Waters, UK) with 2 mL of methanol followed by 2 mL of water with 5 % ammonium hydroxide. Samples were applied to the cartridges without pH adjustment using vacuum and after a 30 min drying step, they were stored in freezer at -18 °C until elution. Elution was made using 2 mL of methanol followed by 2 mL of methanol with 2 % formic acid.

All eluates were evaporated under a stream of nitrogen at 40 °C in a water bath (TurboVap evaporator (Caliper, UK)) and then reconstituted with 250 µL of H_2O:MeOH 80:20. Samples were then injected on the UHPLC-QTOF.

Solid matrix - microwave assisted extraction. The microwave assisted extraction was performed according to Petrie et al., 2015. After collection, digested sludge samples were frozen and then freeze dried using a ScanVac, CoolSafe freeze dryer (Lynge, Denmark). 1 h before the extraction, 50 ng of each internal standard were added to 0.5 g of digested sludge and extraction was performed using 25 mL of 50:50 MeOH:H_2O (pH 2) using a 800 W MARS 6 microwave (CEM, UK). Samples were heated at 110°C for 30 min. After extraction samples were filtered using GF/F filters (0.7 µm) and the content of MeOH was taken to < 5% using H_2O (pH 2). Finally, samples were loaded at 5mL min^{-1} onto Oasis MCX cartridges conditioned with 2 mL of MeOH followed by 2 mL of H_2O (pH 2) at 1 mL min^{-1}. MCX cartridges were then dried for 30 min and analytes were then eluted in separate acidic and basic fractions. For further detail see Petrie et al. (Petrie et al. 2015).

5.4.4 Analysis

Targeted UHPLC-QTOF analysis for selected EDCs: full structural confirmation with commercially available reference standards. The analysis was performed on a Dionex UltiMate3000 UHPLC system (Thermo Fisher UK Ltd.) connected to a maXis HD Q-ToF mass spectrometer (Bruker, UK) and controlled by the Compass software (HyStar™ Bruker, UK). 90 µL injections of analyte standard solutions (see section 2.1) were made and the analytes were separated at a flow rate of 0.4 mL min^{-1} on a BEH C18 column (50 x 2.1 mm, 1.7 µm, Waters UK) using mobile phase A (1 mM ammonium fluoride in water) and mobile phase B (methanol) at the following gradient: 0-3 min 5 %B, 3-4 min 5-60 %B, 4-14 min 60 %B, 14-14.1 min 60-98 %B, 14.1-17 min 98 %B, 17-17.1 min 98-5 %B, 17.1-20 min 5 %B.

Mass calibration of the data was performed by an injection of 10 µL of a calibrant solution (3 parts of 1 M NaOH to 97 parts of 50:50 water:IPA with 2 % FA) at the start of each run before the sample injection. The resulting peak was used for internal mass calibration using the software DataAnalysis (Compass DataAnalysis 4.3 Bruker, UK).

The mass spectrometer was equipped with an ESI source and was operated in both positive and negative ionisation mode. A capillary voltage was set at 4.5 kV, the end plate offset was set to 500 V, a pressure of 3 Bar was used for the nebulizer gas, the drying gas (nitrogen) flow was 11 L min^{-1} and the drying temperature was set at 220

°C. The bbCID settings in negative mode were 0 eV of isCID energy in both MS and MS/MS while the respective collision energies were 7 and 20 eV. The bbCID settings in positive mode were 0 eV of isCID energy in both MS and MS/MS while the respective collision energies were 5 and 20 eV.

Collected data was processed using DataAnalysis and QuantAnalysis.

5.4.5 Method validation

Extraction recovery. Three complementary SPE chemistries (HLB, MCX and MAX) were used to cover as large a spectrum of analytes as possible since the method was to be also used for retrospective analysis of analytes not yet targeted. Oasis HLB sorbents showed the highest SPE recoveries in all studied matrices and therefore HLB sorbent was selected for further study. Due to co-elution, 2,4,5-trichlorophenol and 2,4,6-trichlorophenol were evaluated together as one peak. The method extraction recovery was evaluated by spiking 100 mL of influent and effluent wastewater and river water in triplicate at two different analyte concentrations: 100 and 200 ng L-1 and internal standards: 100 ng L-1 after SPE. SPE recoveries were calculated as corrected recoveries (i.e. taking the internal standard concentration into consideration) by the ratio of the concentration of target analytes in wastewater solutions when spiked before SPE (minus the concentration of analyte in the blank wastewater sample), divided by the standard mobile phase concentration (Equation 1).

$$SPE\ Recoveries_{corrected} = \left(\frac{A_{spiked\ before\ SPE} - A_{blank}}{A_{mobile\ phase}}\right) x\ 100\ \% \qquad \text{Equation 1}$$

Choice of internal standards. Internal standards for each analyte were chosen based on 4 different criteria. If an isotopically labelled analogue was available, then that was the internal standard of choice. If a labelled standard was not available, then the internal standard was chosen based on both similarities in physicochemical properties of analytes and similar behaviour during SPE and LCMS analysis.

Linearity. The linearity in detector's response for each analyte was evaluated by the construction of calibration curves from thirteen different concentration levels (ranging from 0.01 µg L^{-1} to 100 µg L^{-1}) in mobile phase. The linearity was interpreted as the R^2 for the resulting linear regressions (based on all concentration levels or a selection thereof depending on the analyte).

Instrument and method limits of detection and quantification. The instrument detection and quantification limits (IDL and IQL respectively) were evaluated through the use of the calibration curves to calculate the concentrations that gave signal-to-noise ratios of 3 and 10 respectively. However for some compounds the IQL was found to be at a concentration that was below the linear range, in this case the lowest point of the calibration curve was selected as IQL. The IDLs and IQLs were then used to calculate the method detection and quantification limits (MDL and MQL respectively) by using Equation 2.

$$ML = \frac{IL}{400} * \frac{1}{RC}$$

Equation 2

ML and IL stand for MDL/MQL and IDL/IQL respectively. 400 is the concentration factor due to SPE and RC is the SPE recovery. When the calculated MQL was lower than the lowest concentration used in the calibration curve then the MQL was set to match the calibration curve.

Inter- and intraday precision. Inter- and intraday precision for the instrument was evaluated from QC-standards up to three concentrations (0.1, 5 and 100 µg L^{-1}) injected in triplicate. Standards that were within the linear range of the analyte were used for the calculations. The inter- and intraday precision for the method was evaluated by spiking wastewater and river water at two different concentrations (100 and 200 ng L^{-1}) followed by extraction in triplicate on three consecutive days (total n=9) using HLB cartridges.

Accuracy. The accuracy was assessed by comparing calculated concentrations using established calibration curves with the theoretical concentrations and calculating the average error with its associated RSD.

5.4.6 Post-acquisition data mining for metabolite identification and quantification

The collection of full-scan spectra permits to measure compounds without previous compound-specific tuning with the possibility of retrospective data analysis, and the capability of performing structural elucidations and quantification of unknown or suspect compounds. A level system approach utilised in this paper to identify and

quantify metabolites with different levels of confidence was modified from Schymanski et al.,(Schymanski et al. 2014). Two confidence levels were investigated (level 1a and 1b) with minimum identification criteria required being: I) lower retention time then their respective parent compound given their lower lipophilicity; II) high mass accuracy (mass error below 10 ppm); III) isotope pattern matching the predicted one (within 5% error).

Level 1a: Confirmed structure by commercially available reference standards followed by full quantification. The proposed structure of metabolites was confirmed via the utilisation of a reference standard with both MS and MS/MS mode and matching retention time.

Level 1b: Confirmed structure by reference compound synthesised *in vitro*. An exact structure of metabolites has been proposed using *in vitro* HLM/S9 fraction assays (see Lopardo et al. (Lopardo et al. 2017) for details) as evidence. The proposed structure of metabolites was confirmed by comparing both MS and MS/MS mode and matching retention time to the *in vitro* produced metabolite as well as *in vivo* products in pooled urine and wastewater.

5.5 Results and discussion

5.5.1 Method development

The aim of this manuscript was to develop a sensitive and selective multi-residue method for both quantification of trace concentrations of 37 EDCs in wastewater and in the receiving environment and a posteriori analysis of metabolites of interest. The workflow of the developed method is presented in Fig 5.1.

5.5.2 UHPLC-QTOF method development

Mobile phase composition. Initial experiments revealed that mobile phase composed of 0.1 % FA in water and methanol (linear gradient) did not provide satisfactory separation of all analytes and did not facilitate satisfactory signal intensities in negative ionization mode (e.g. BPA). On the other hand, 1 mM NH_4F in water as the aqueous phase resulted in better signal intensities in negative ionisation mode but it also resulted in lower signal intensities in positive ionization mode. As the aim was to have one separation method serving both positive and negative ionisation modes, the gain in signal intensities in negative mode was weighed against the loss in signal intensities in positive mode. Since more analytes were detected using mobile phase containing 1 mM NH_4F and the gain in signal intensities in negative mode was higher than the number of analytes and the loss of signal in positive mode, this mobile phase was selected for further experiments. In order to achieve best separation of analytes several mobile phase gradients were trialled. Although HRMS can be used to detect and quantify co-eluting analytes without problems, in many cases it was expected that the complex matrix of wastewater could cause problems if too many analytes co-eluted. Several different gradients were therefore developed and tested to separate as many of them as possible using a 20 min long programme. By using a hold time of three minutes at the starting condition of 5 % MeOH (B) followed by quickly ramping up to 60 % B and then holding there for 10 minutes it was possible to get separation both of low to mid non-polar compounds eluting during the first half of the run and of more non-polar compounds that were eluting at the end of the run. Without the long hold time at the start and the middle of the run the resolution within each group of compounds of similar polarity would be inadequate due to the expected interferences of the sample matrix. The final mobile phase composition was therefore as follows: mobile phase A (1 mM NH_4F in water) and mobile phase B (methanol) at the following gradient: 0-3 min 5 %B, 3-4 min 5-60 %B, 4-14 min 60 %B, 14-14.1 min 60-98 %B, 14.1-17 min 98 %B, 17-17.1 min 98-5 %B, 17.1-20 min 5 %B).

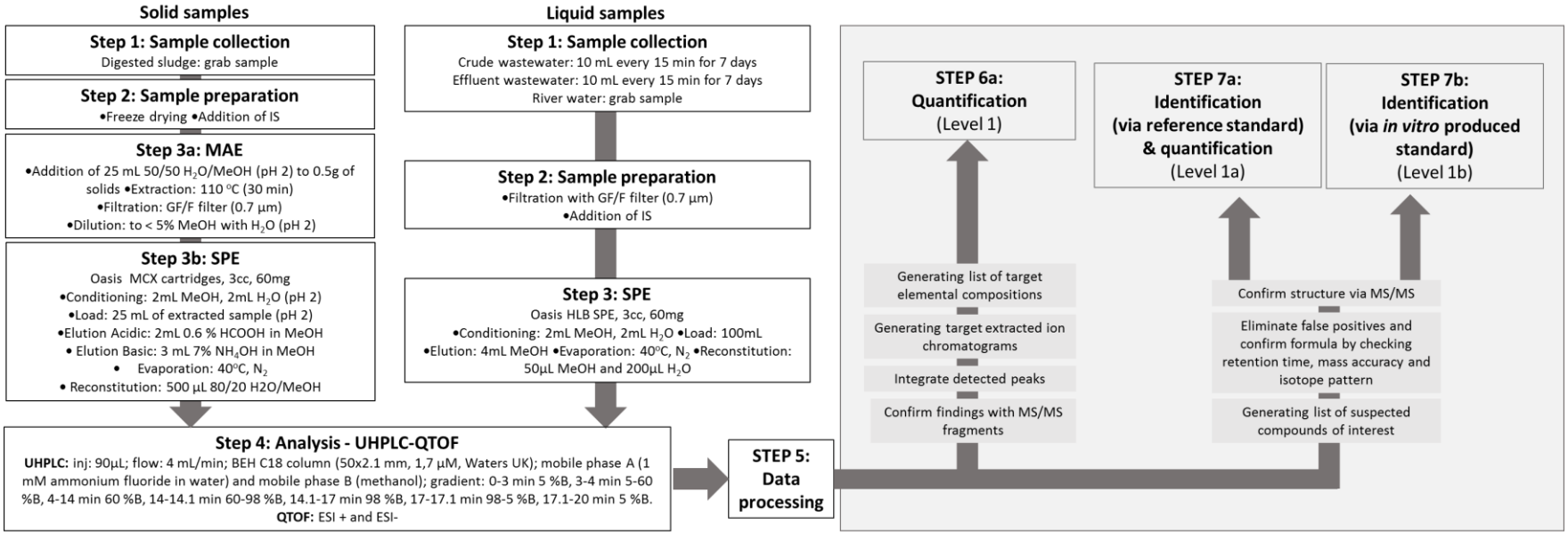


Figure 5.1 Schematic overview of the analytical protocol used to investigate the presence of EDCs in environmental matrices.

Table 5.1 UHPLC-QTOF instrument performance parameters

Analyte	Internal standard used	ESI	Rt [min]	Linearity		Intra-day instrument performance[1, 2]		Inter-day instrument performance		IDL [µg L^{-1}]	IQL [µg L^{-1}][3]
				Range [µg L^{-1}]	R^2	Accuracy [%]	Precision [%]	Accuracy [%]	Precision [%]		
2,4,5 & 2,4,6-trichlorophenol	Triclosan-d3	neg	9.1	0.28-69.5	0.9989	92.6	8.5	90.1	12.7	0.081	0.27
2-ethylhexanoic acid*[4]	Triclosan-d3	neg	7.9	4-36.5	0.9978	/	/	/	/	/	/
2-naphthol	Bezafibrate-d6	neg	7.2	0.09-36	0.9985	95.7	10.8	77.8	17.9	0.027	0.09

4,4'-dihydroxybenzophenone	Bezafibrate-d6	neg	6.6	0.008-21.5	0.9994	109.2	11.4	95.2	7.8	0.024	0.08
4-benzylphenol	Bezafibrate-d6	neg	8.3	0.1-55	0.9995	138.8	9.8	136.6	19.4	0.030	0.1
4-chloro-3,5-dimethylphenol	4-Cl-3-methylphenol-d2	neg	8.2	0.04-27.5	0.9995	103.4	11.8	109.7	5.1	0.012	0.04
4-chloro-3-methylphenol	4-Cl-3-methylphenol-d2	neg	7.5	0.064-27.5	0.9987	120.2	2.4	120.2	3.5	0.019	0.064
4-n-nonylphenol	Triclosan-d3	neg	17.3	0.6-63	0.9984	123.71	4.8	124.1	8.1	0.180	0.6
4-n-octylphenol*	4-Cl-3-methylphenol-d2	neg	17	0.095-56	0.9954	/	/	/	/	/	/
Atrazine	Atrazine-d5	pos	7.5	9.9-99	0.9984	102.1	1.6	102.1	3.3	0.010	0.034
Benzophenone-1	4-Cl-3-methylphenol-d2	neg	7.8	0.11-31.8	0.9975	110.4	8.3	110.4	15.0	0.033	0.11
Benzophenone-2	Bezafibrate-d6	neg	6.8	0.12-88	0.9996	98.0	6.5	98.0	10.1	0.036	0.12
Benzophenone-3	BP-3-d5	pos	9.2	0.59-54	0.9992	104.4	8.4	113.5	4.2	0.177	0.59
Benzophenone-4	Bezafibrate-d6	neg	6.5	0.14-11	0.9972	98.5	5.1	98.5	19.5	0.042	0.14
Benzylparaben	Bezafibrate-d6	neg	8.3	0.07-33	0.9998	74.8	8.9	74.8	19.3	0.021	0.07
Bisphenol A	4-Cl-3-methylphenol-d2	neg	7.7	0.28-28.5	0.9972	114.8	2.5	114.8	9.4	0.084	0.28
BADGE-2-Cl	4-Cl-3-methylphenol-d2	neg	13.1	0.1-4.7	0.9989	103.4	7.1	103.4	19.9	0.030	0.1
Butylparaben	Bezafibrate-d6	neg	8.3	0.13-32	0.9994	93.4	8.8	93.4	15.6	0.039	0.13
Chlorothymol	Triclosan-d3	neg	10.7	0.1-48.5	0.9974	118.0	2.8	135.8	12.3	0.031	0.1
dibutyl phthalate	Atrazine-d5	pos	13.8	0.96-96	0.9899	81.3	3.0	80.5	3.3	0.288	0.96
Ethyl paraben	Bezafibrate-d6	neg	7.0	0.27-100.5	0.9993	92.9	5.7	92.9	9.0	0.081	0.27
Galaxolide	Atrazine-d5	pos	17.1	0.45-51.5	0.9967	94.6	16.3	91.8	18.0	0.135	0.45
HBCD*	Bezafibrate-d6	neg	17.5	0.08-10.5	0.9966	/	/	/	/	/	/
MEHP	4-Cl-3-methylphenol-d2	neg	8.7	0.27-5.1	0.9952	90.4	7.4	90.4	19.6	0.081	0.27
Methylparaben	Bezafibrate-d6	neg	6.6	0.35-30.5	0.9990	131.7	10.8	131.7	16.3	0.105	0.35
Monobutyl phthalate	4-Cl-3-methylphenol-d2	neg	6.7	0.16-39	0.9969	130.3	16.4	130.3	19.0	0.048	0.16
Musk ketone	4-Cl-3-methylphenol-d2	neg	12	0.53-27.5	0.9952	129.9	9.1	94	12.2	0.159	0.53
Padimate O	Atrazine-d5	pos	17.3	0.39-27.4	0.9974	93.6	14.7	86.8	16.9	0.117	0.39
Perfluorooctanesulfonic acid	Triclocarban-d4	neg	8.9	0.096-53	0.9993	98.9	7.2	99.2	18.6	0.288	0.96

Perfluorooctanoic acid	Triclocarban-d4	neg	8.2	1.37-104.5	0.9993	117.3	2.7	117.3	4.1	0.411	1.37
PBSA	Triclosan-d3	neg	6.1	0.16-11	0.9974	94.0	5.8	94.0	20.0	0.048	0.16
Prochloraz*	BP-3-d5	pos	11.6	0.3-12.7	0.9970	122.1	14.4	133.9	15.4	0.090	0.3
Propylparaben	4-Cl-3-methylphenol-d2	neg	7.5	0.38-31.5	0.9996	122.0	6.3	122.0	15.7	0.114	0.38
Tetrabromobisphenol A	Triclocarban-d4	neg	15.6	0.144-12	0.997	171.2	9.3	171.2	9.4	0.043	0.144
Triclocarban	Triclocarban-d4	neg	13.5	0.11-27.5	0.9993	114.5	3.5	114.5	4.6	0.033	0.11
Triclosan	Triclosan-d3	neg	14.6	0.11-53.2	0.9997	102.1	2.9	102.1	7.0	0.033	0.11
Vinclozolin	4-Cl-3-methylphenol-d2	neg	11.2	1.1-88	0.9972	104.8	14.4	104.8	19.4	0.330	1.1
Solid analysis											
2,4,5 & 2,4,6-trichlorophenol	4-Cl-3-methylphenol-d2	neg	9.1	0.28-69.5	0.9980	83.2	8.1	83.2	19.0	0.084	0.28
4-n-nonylphenol	4-Cl-3-methylphenol-d2	neg	17.3	0.63-63	0.9972	123.71	4.8	128.3	11.6	0.190	0.63
Chlorothymol	4-Cl-3-methylphenol-d2	neg	10.7	0.25-48.5	0.9971	118.7	3.0	138.3	14.5	0.075	0.25
PBSA	4-Cl-3-methylphenol-d2	neg	6.1	0.27-11	0.9969	111.9	6.0	94.1	19.5	0.081	0.27
Triclosan	4-Cl-3-methylphenol-d2	neg	14.6	0.29-53	0.9997	122.8	2.8	122.8	7.8	0.090	0.29

*Note: **1:** concentration levels: 0.1, 5 and 100 ng/mL used for inter- intraday precision and accuracy (only the levels within the linear range were used for each compound). **2:** 5 replicates were injected of each concentration level for each day. **3:** IQL is set as the lowest linear point that has a precision of <±20 %. 4; starred compounds denoted quality control criteria out of the acceptance range suggested by the European commission*

Mass spectrometry parameters. MS/MS parameters were optimised for 37 analytes and their corresponding labelled internal standards (for details see Table 5.1). Of the 37 compounds investigated only 6 denoted better sensitivity in ESI positive mode, while the vast majority provided better sensitivity in ESI negative mode. One SRM transition and high mass accuracy (< 10ppm mass error) for quantifier, qualifiers and isotope ions were used as criteria for identification and quantification purposes according to the EU guidelines (European commission 2002). For all compounds the molecular mass plus/minus a hydrogen ion was selected as quantifier ion, while one SRM transition was monitored in complex matrices for confirmation purposes. Unfortunately, due to limited fragmentation, no SRM transition could be monitored for 3 compounds (BADGE-2-Cl, HBCD and triclosan). Nonetheless the level of certainty for those compounds is still high due to the presence of multiple halogens in their chemical structures, meaning complex and highly distinctive isotope patterns. Retention time and ion ratio within the standard tolerance were also monitored to ensure the quality of the data. Examples of two compounds (methylparaben and triclosan) identification in environmental matrices is reported in Figure 5.2 and 5.3.

Solid-phase extraction. Three complementary SPE chemistries (HLB, MCX and MAX) were used to cover as large a spectrum of analytes as possible since the method was to be also used for retrospective analysis of analytes not yet targeted. SPE recoveries were evaluated in influent wastewater at two different levels of spiking. The observed recoveries are shown in Table 5.2. Due to co-elution, 2,4,5-trichlorophenol and 2,4,6-trichlorophenol were evaluated together as one peak.

Choice of IS. Not all analytes were available as isotope labelled standards that could be used as internal standards. For those analytes that did not have an isotope labelled analogue the IS was chosen by evaluating several labelled compounds in terms of extraction efficiency, retention times and analyte vs internal standard area ratios stability in pure water and wastewater. The internal standards chosen are presented in Table 5.1.

Table 5.2 SPE-UHPLC-QTOF method performance parameters

Analyte	Wastewater influent			Wastewater effluent			River water			Digested sludge		
	SPE recovery [%][1]	MDL [ng L^{-1}]	MQL [ng L^{-1}]	SPE recovery [%]	MDL [ng L^{-1}]	MQL [ng L^{-1}]	SPE recovery [%]	MDL [ng L^{-1}]	MQL [ng L^{-1}]	MAE recovery [%]	MDL [ng g^{-1}]	MQL [ng g^{-1}]
2,4,5 & 2,4,6-trichlorophenol	94.4	0.222	0.741	89.5	0.235	0.782	101.9	0.206	0.687	66.5	0.31	1.05
2-naphthol*[3]	140.7	0.063	0.211	91.4	0.074	0.246	109.8	0.061	0.205	/	/	/
4,4'-dihydroxybenzophenone	106.6	0.057	0.190	100.6	0.060	0.199	109.5	0.055	0.183	59.7	0.01	0.033
4-benzylphenol*	105.2	0.083	0.275	80.1	0.094	0.312	100.9	0.074	0.248	/	/	/
4-chloro-3,5-dimethylphenol	90.8	0.037	0.123	80.7	0.037	0.124	101.5	0.030	0.099	131.9	0.02	0.076
4-chloro-3-methylphenol	81.5	0.097	0.326	89.9	0.053	0.178	105.6	0.045	0.152	68.9	0.07	0.232
4-n-nonylphenol	49.1	0.882	2.941	43.8	1.027	3.425	67.7	0.665	2.216	18.4	2.5	8.176
Atrazine	99.0	0.025	0.086	94.2	0.027	0.090	102.6	0.024	0.083	68.4	0.01	0.036
Benzophenone-1	89.7	0.092	0.307	107.9	0.076	0.255	102.0	0.081	0.270	69.2	0.1	0.398
Benzophenone-2	102.9	0.087	0.292	98.9	0.091	0.303	106.6	0.084	0.281	75.5	0.1	0.397
Benzophenone-3	86.1	0.514	1.714	80.4	0.550	1.835	87.2	0.507	1.692	186.2	0.2	0.792
Benzophenone-4	105.3	0.100	0.332	80.1	0.131	0.437	105.1	0.100	0.333	17.7	0.6	1.977
Benzylparaben	122.4	0.043	0.143	100.1	0.052	0.175	99.7	0.053	0.176	145.9	0.03	0.120
Bisphenol A	100.4	0.209	0.697	64.2	0.327	1.090	86.0	0.244	0.814	95.7	0.2	0.731
BADGE-2-Cl	122.9	0.061	0.203	73.5	0.102	0.340	81.8	0.092	0.306	24.6	0.3	1.02
Butylparaben*	105.3	0.093	0.309	95.1	0.103	0.342	107.4	0.091	0.303	/	/	/
Chlorothymol	84.5	0.222	0.739	88.4	0.212	0.707	96.1	0.195	0.650	101.4	0.2	0.616
Dibutyl phthalate*	71.2	1.012	3.372	93.6	0.769	2.564	95.1	0.757	2.524	/	/	/

Ethylparaben	87.9	0.230	0.768	108.4	0.187	0.623	107.1	0.189	0.630	112.1	0.2	0.602
Galaxolide*	50.7	0.666	2.219	100.8	0.335	1.116	102.1	0.331	1.102	/	/	/
MEHP*	110,5	0.183	0.611	82.4	0.246	0.819	95.6	0.212	0.706	61.6	0.3	1.1
Methylparaben	80.5	0.326	1.086	109.3	0.240	0.801	104.6	0.251	0.837	86.4	0.3	1.013
Monobutyl phthalate	84.2	0.143	0.475	84.9	0.141	0.471	87.1	0.138	0.459	26.3	0.5	1.52
Musk ketone*	73.9	0.538	1.793	72.1	0.551	1.838	81.7	0.487	1.622	/	/	/
Padimate O*	49.8	0.587	1.957	28.8	1.016	3.385	53.7	0.545	1.816	/	/	/
Perfluorooctanesulfonic acid*	89.6	0.708	2.360	84.7	0.850	2.834	69.1	1.042	3.473	/	/	/
Perfluorooctanoic acid	101.7	1.147	3.825	92.3	1.113	3.711	95.4	1.077	3.590	18.4	5.6	18.6
PBSA	138.5	0.087	0.289	73.5	0.163	0.544	71.4	0.115	0.385	20.1	0.6	1.99
Prochloraz*	95.7	0.235	0.784	83.8	0.268	0.895	107.0	0.210	0.701	/	/	/
Propylparaben	89.2	0.319	1.065	112.9	0.252	0.841	93.5	0.305	1.016	86.4	0.3	1.099
Tetrabromobisphenol A	88.3	0.122	0.408	71.0	0.151	0.507	83.8	0.128	0.430	11.3	1.0	3.176
Triclocarban*	70.7	0.117	0.389	61.9	0.133	0.444	73.4	0.112	0.375	/	/	/
Triclosan*	73.8	0.112	0.373	80.4	0.103	0.342	82.8	0.100	0.332	/	/	/
Vinclozolin*	33.0	2.500	8.333	5.0	16.50	55.00	4.99	16.533	55.110	/	/	/

*Note: **1:** based on triplicate extractions. **2:** based on triplicate injections at three concentration levels (n=9), for some analytes one injection had to be removed making n=8 **3:** starred compounds showed poor or no recovery from solid samples*

5.5.3 MAE/SPE -UHPLC-QTOF method validation

Solid-phase extraction. HLB gave good relative recoveries for most compounds with the 54 % of analytes having recoveries between 80 and 110 % (Table 5.2). The difference in recoveries between high and low spiking levels was mostly below 10 % RSD. For MCX the relative recoveries were between 70 and 110 % for half of the analytes. The relative recovery difference between high and low spiking levels was slightly higher with 27 of the analytes having an RSD % below 14 %. MAX gave the largest spread for recoveries with half of the analytes having relative recoveries between 60 and 120 %. For 25 of the analytes the RSD % for the two different levels of spiking was below 12 %. HLB recoveries were also evaluated at two different levels of spiking in effluent wastewater and river water. Relative recoveries for effluent wastewater were in between 80 % and 110 % for 68 % of the compounds with relative recovery difference between high and low spiking level mostly below 10 % RSD. Relative recoveries were better when SPE was performed on river water samples due to the lower complexity of the matrix. 84 % of the values were between 80 and 110 % with relative recovery difference between high and low spiking level mostly below 10 % RSD. Due to the best performance, HLB sorbents were chosen for further method development and validation.

MAE/SPE recoveries. A microwave assisted extraction (MAE) method developed by Petrie et al. (Petrie et al. 2015) was selected to prepare solid samples as it provided good recoveries for over 60 of the 90 compounds investigated in the study, including some endocrine disruptors selected in the present work such as BP-1, BP-2, BP-4, BPA, triclosan and methyl-, ethyl-, propyl-, and butylparaben. In this study, MAE/SPE recoveries ranged from 11 to 186 % with the majority of compounds denoting recoveries between 59 and 115 % (Table 5.2). However, 8 compounds could not be analysed with the current method due to poor SPE recoveries, poor MAE efficiency or a combination of the two. More specific targeted analytical methods are therefore necessary for these EDCs.

Inter and intra-day accuracy and precision. Intra and inter-day accuracy was typically within the range 80–130% for most chemicals both within the same day and between different days (Table 5.1). Instrumental intra and inter-day precision calculated for three concentration levels at three consecutive days denoted on average 8% and 13%

(Table 5.1). The precision of the method was also evaluated at three different concentration levels that were extracted in triplicate using HLB on three consecutive days (giving a total n=9). The results from the evaluation are presented in Table 5.2.

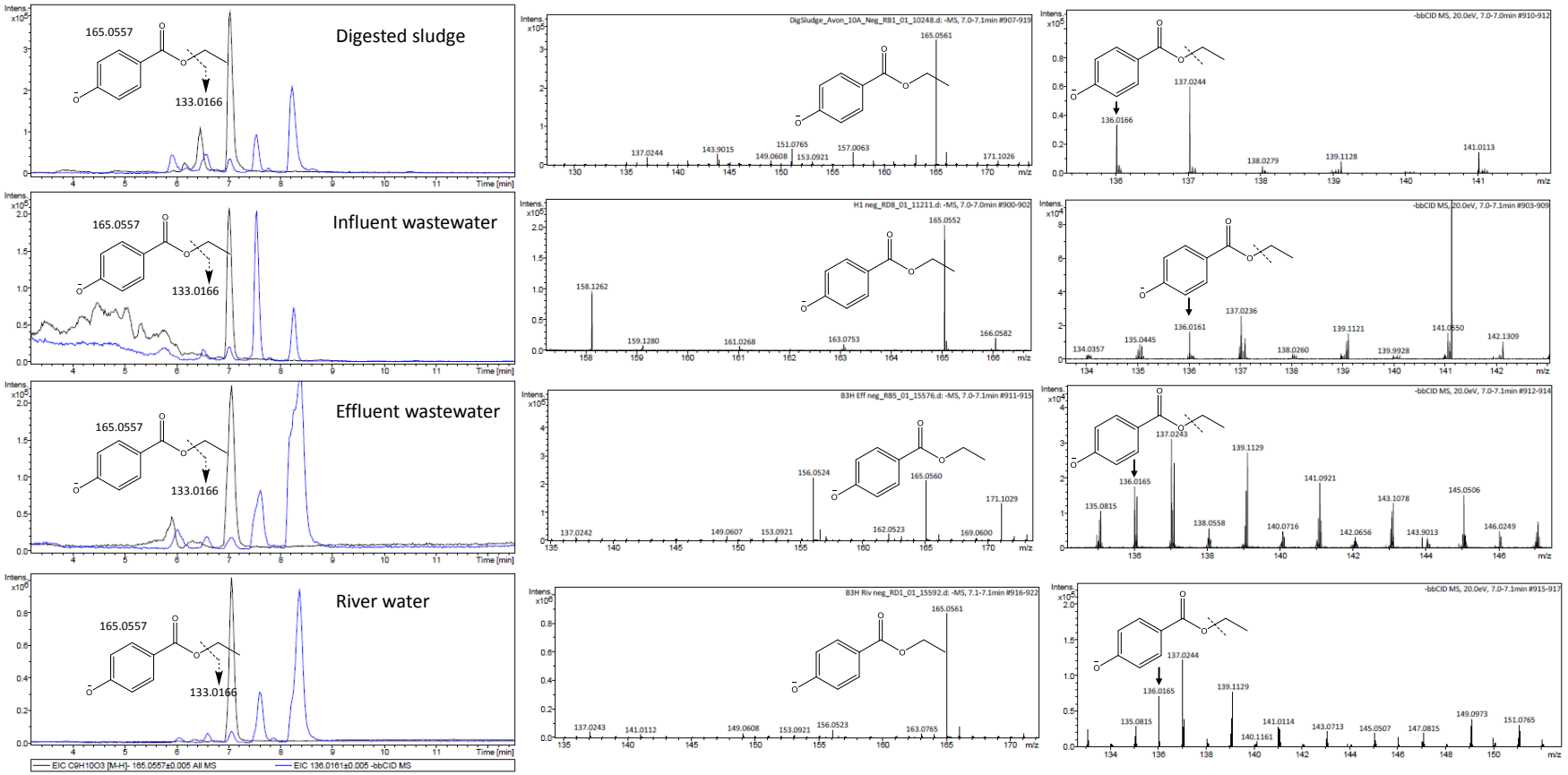


Figure 5.2 Separation and identification of ethylparaben in all analyzed matrices (influent and effluent wastewater, river water and digested sludge). XIC at m/z 165.0557 (0.005-Da mass-window width, black trace) and at m/z 136.0166 (0.005-Da mass-window width, blue trace) in four different matrices (from top to bottom), and respective low energy and high energy mass spectra (from left to right) of the peak eluted at 7.0 minutes.

Table 5.3 Environmental data week average

Analyte	Wastewater influent (ng L^{-1})	Wastewater effluent (ng L^{-1})	River upstream (ng L^{-1})	River downstream (ng L^{-1})	Digested sludge (ng g^{-1})
2,4,5- & 2,4,6-trichlorophenol	2.5±1.4	2.4±1.0	0.1±0.3	0.04±0.16	57.5±3.7
2-ethylhexanoic acid*	17612.3±21685.4	417.0±236.1	4290.5±2277.5	3030.9±1594.6	n.a.
2-naphthol	78.0±20.5	9.3±6.4	7.9±9.2	6.7±8.0	n.a.
4,4'-dihydroxybenzophenone	24.2±4.2	15.2±1.5	1.7±0.4	2.0±0.4	3.2±0.19
4-benzylphenol	10.4±6.0	18.2±9.2	43.0±64.9	28.3±38.7	n.a.
4-chloro-3,5-dimethylphenol	8492.4±3344.6	3975.2±932.8	34.7±13.7	100.8±29.5	455.6±7.5
4-chloro-3-methylphenol	2632.9±903.7	43.3±16.2	4.4±1.5	3.3±0.9	520.5±16.6
4-n-nonylphenol	< MQL	31.2±16.5	12.1±7.9	13.3±6.5	3.4±0.06
4-n-octylphenol	n.d.	n.d.	n.d.	n.d.	n.d.
Atrazine	48.3±4.1	45.3±6.7	66.6±5.2	59.5±9.6	n.d.
Benzophenone-1	47.1±14.6	6.8±1.5	0.6±0.3	0.8±0.2	8±0.6
Benzophenone-2	13.1±2.5	10.6±0.9	0.9±0.6	0.9±0.7	3.8±0.4
Benzophenone-3	753.0±129.2	44.7±7.2	32.4±34.1	4.5±11.7	42.4±5
Benzophenone-4	2711.5±808.1	660.9±157.7	41.6±19.4	55.3±27.9	33.1
Benzylparaben	n.d.	n.d.	n.d.	n.d.	0.44±0.07
Bisphenol A	812.5±52.2	23215.9±21945.31	14.3±8.5	12.9±3.4	15690±542
BADGE-2-Cl	0.9±0.67	0.31±0.3	0.73±0.38	0.77±0.43	3.2±0.07
Butylparaben	11.1±4.9	1.3±0.2	1.4±0.7	1.7±0.6	n.a.
Chlorothymol	n.d.	n.d.	n.d.	n.d.	5.6±0.1
Ethylparaben;	143.3±23.2	1.1±0.3	2.6±3.5	1.2±1.3	5.34±1.5
Galaxolide	164.5±40.2	50.2±18.7	11.0±4.2	9.4±2.7	n.a.
HBCD*	1.29±0.84	0.73±0.33	0.93±0.88	0.57±0.49	6.03±1
Methylparaben	749.7±136.8	2.8±0.7	9.7±9.1	5.8±7.1	88.8±2.7

Musk ketone	n.d.	n.d.	n.d.	n.d.	n.a.
Padimate O	140.4±42.0	94.6±32.3	139.8±147.8	248.5±157.1	n.a.
Perfluorooctanesulfonic acid	4.5±1.0	7.5±2.8	11.9±3.1	12.8±3.7	n.d.
Perfluorooctanoic acid	10.0±6.7	12.3±7.6	12.6±3.6	12.1±4.5	4±0.7
PBSA	2152.1±484.1	1606.0±473.9	3655. 4±5504.6	298.2±100.6	162±27.7
Prochloraz	100±62	5±4.5	35.3±32.8	22±20.1	n.a.
Propylparaben	143.1±23.3	4.7±0.7	1.5±1.2	1.4±1.8	4.4±0.5
Tetrabromobisphenol A	13.9±11	1.67±0.32	n.d.	n.d.	25.1±1.4
Triclocarban	17.1±5.1	8.5±1.6	4.7±3.4	3.4±2.0	n.a.
Triclosan	589.0±59.3	133.2±14.6	19.8±10.0	18.7±6.2	n.a.
Vinclozolin	n.d.	n.d.	n.d.	n.d.	n.a.

*Note: **1:** based on triplicate extractions. **2:** based on triplicate injections, for some analytes one injection had to be removed making n=8 **3:** n.a. non analyzed as compounds showed poor or no recovery from solid samples; *- results are only semi-quantitative dues to poor accuracy and precision.*

Table 5.4 UHPLC-QTOF instrument and method performance parameter and BPA sulphate concentration in wastewater

Analyte	IS	Linearity Range [µg L^{-1}]	R^2	Accuracy[1] [%]	Precision[1] [%]	IDL [µg L^{-1}]	IQL [µg L^{-1}]	**Wastewater SPE recovery [%]****	**MDL [ng L^{-1}]**	**MQL [ng L^{-1}]**	**Average concentration in wastewater [ng L^{-1}]*****
Bisphenol A Sulphate	4-chloro-3-methylphenol-d2	1.39 - 103.4	0.9972	98.3	2.1	0.41	1.39	63.7±6.3	0.016	0.055	2663.9±422

**concentration levels: 0.1, 5 and 100 ng/mL used for precision and accuracy; ** based on duplicate extractions at two concentration levels; ***based on wastewater samples collected over seven days*

Overall, the intraday precision of the method is good with the majority of analytes giving precision values below 10 % RSD in both positive and negative mode with the highest value being 18.4 % RSD. For inter-day precision, the spread was a little bit higher with all analytes giving less than 20 % RSD. Three compounds (2-ethylhexanoic acid, 4-n-octylphenol, HBCD) need to be discussed separately since their calibration curves showed satisfactory linearity across a good range of concentrations but accuracy and precision did not meet the criteria established by the European Commission. The high variability was due to poor ionization rate in the selected analytical conditions. Their analysis can therefore be considered only qualitative. *Detection and quantification limits.* The method was developed to accommodate both negative and positive ESI polarity with the same mobile phase. Because the majority of the compounds ionised better in negative ionisation mode the mobile phase was geared towards negative ionisation. This can be observed in the instrumental detection and quantification limits that are in the low ng L^{-1} (between 10 and 50 ng L^{-1}) for most compounds analysed in negative mode while the compounds analysed in positive mode have IDL and IQLs in the hundreds of ng L^{-1} (Table 5.1 and 5.2).

Linearity range. A set of 13 calibration solutions containing all analytes and internal standards were made up at the following concentrations: 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 ng mL^{-1}. These solutions were analysed and integrated using the QuantAnalysis software. For each analyte the analyte/internal standard area ratio for the thirteen calibration levels were compared and investigated by drawing up calibration curves. The R^2-value for the resulting calibration curves is presented alongside their linear range in Table 5.1. Most analytes showed good linearity (interpreted from the R^2) with varying linear ranges (from two to three orders of magnitude) highly dependent on individual analytes.

5.5.4 Targeted analysis of environmental samples with MAE/SPE-UHPLC-QTOF including full structural confirmation with commercially available reference standards

Environmental samples collected over one-week long sampling campaign were subjected to both quantitative and qualitative analysis. A summary for the quantified analyte levels in five different matrices is shown in Table 5.3**.** Several analytes were

present at very high concentration levels in incoming and effluent wastewater that did not require any extraction steps to be undertaken (Table S5.3).

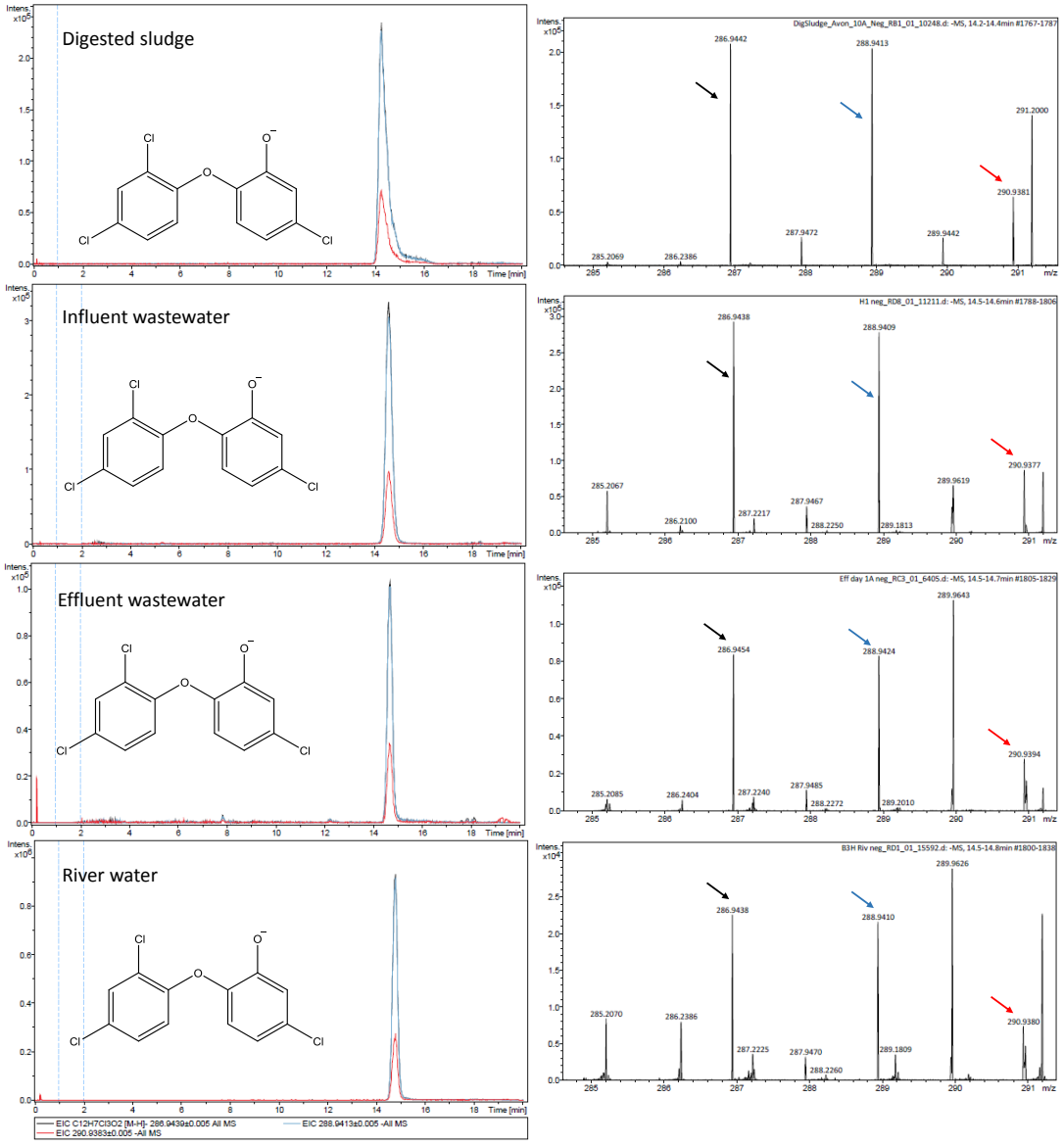


Figure 5.3 Separation and identification of triclosan in all analyzed matrices (influent and effluent wastewater, river water and digested sludge). XIC at m/z 286.9439 (0.005-Da mass-window width, black trace), at m/z 288.9413 (0.005-Da mass-window width, blue trace) and at m/z 290.9383 (0.005-Da mass-window width, red trace) in four different matrices (from top to bottom). The right column shows the mass spectra of the peak eluted at 14.6 minutes and the black, blue and red arrows indicate respectively $[M]^-$, $[M+2]^-$, $[M+4]^-$ peaks with relative intensities matching those expected from a compound with three chlorines within 5% of the predicted abundance.

Other compounds showed an irregular occurrence pattern. For example, phenylbenzimidazolesulfonic acid (also known as Ensulizole or PBSA) was detected at high levels (>1000 ng L^{-1}) in influent and effluent wastewater with constant occurrence patterns. However, a spike in its concentration was observed on two consecutive days within the sampling week in the river water resulting in increased levels reaching 4000 ng L^{-1}. This could be due to (accidental) disposal of larger quantities of PBSA upstream from the sampling point.

Interesting was also the low presence (< MQL) of the surfactant 4-n-nonylphenol in influent wastewater while its concentration in effluent wastewater was calculated higher than > 30 ng L^{-1}. That might be due to its formation during wastewater treatment as a result of decomposition of nonylphenol polyethoxylates (Minamiyama et al. 2006). Similar pattern was observed for BPA whose concentrations were significantly higher in effluent rather than influent wastewater. It is suggested that the increase in concentration might be due to *in situ* degradation of conjugated metabolites (i.e. glucuronidates) as it has been previously observed for other compounds (Leclercq et al. 2009). Nonetheless in both cases further investigation is required.

Out of the 37 compounds investigated only 3 compounds (n-octylphenol, musk ketone, vinclozolin) were not detected in studied environmental matrices, likely due to low sensitivity of the method towards some of these compounds.

Significant concentrations of 22 endocrine disruptors were also found when analysing solid samples (digested sludge). Concentrations ranged from 0.44 ng g^{-1} for benzylparaben to 15 µg g^{-1} for BPA. The observation of benzylparaben, along with chlorothymol, was of particular interest given that these compounds were not detected in wastewater and river water samples, highlighting the importance of investigating the presence of chemicals in solid matrices alongside water samples.

5.5.5 Post-acquisition data mining for metabolite identification and quantification

The main advantage of LC-HRMS is the possibility of identifying compounds which were not included in the initial analysis (post-target or retrospective analysis) that can be achieved without the need for re-extraction (meaning reduction in the use of

solvents, reagents and materials). This possibility enables the investigation of newly identified compounds that are not yet integrated into the monitoring strategies currently in use such as compound's metabolites (e.g. BPA sulphate and 4-Cl-3-methylphenol sulphate).

As discussed in Experimental, the level system approach utilised in this paper to identify and quantify metabolites with different levels of confidence was modified from Schymanski et al.(Schymanski et al. 2014). Two confidence levels were investigated:

Level 1a: Confirmed structure by commercially available reference standards followed by full quantification (BPA sulphate, metabolite of BPA).

Level 1b: Confirmed structure by reference compound synthesised *in vitro* (4-Cl-3-methylphenol sulphate, metabolite of 4-Cl-3-methylphenol).

5.5.6 Confirmed metabolite structure by commercially available reference standards followed by full quantification

From analyses previously carried out the signal at m/z 307.0646, corresponding to bisphenol A sulphate (elemental formula $C_{15}H_{16}O_5S$ with a mass error of 9 ppm), was extracted from the total ion current of each wastewater samples chromatogram (Figure 5.4) and a chromatographic peak at 6.8 min was found in all the samples analysed. Retention time was expectedly lower in the used chromatographic conditions (reverse phase) due to the metabolite higher polarity than the parent compound. BPA was in fact eluted after 7.7 min. In order to confirm that the chromatographic peak corresponds with the BPA sulphate, the workflow developed by Lopardo et al., (Lopardo et al. 2017) was employed and mass error, and a high-resolution MS/MS spectrum (Figure 5.4) were used. A further level of confidence was added when results were compared to a reference standard reaching the highest level of confidence according to the approach suggested by Schymanski et al.,(Schymanski et al. 2014).

Subsequently a calibration plot (R^2 = 0.997) at eight concentration levels (each one replicated three times) ranging from the LOQ (1.39 ng mL^{-1}) to 103.4 ng mL^{-1} was used to quantify BPA sulphate. LOD and LOQ were expressed as the concentration of BPA sulphate that give a signal to noise ratio of 3 and 10. Once HLB recoveries were assessed following the same protocol described in section 2.3.1 method detection and

quantification limits (MDL and MQL) were established as respectively 0.016 and 0.055 ng L^{-1}. BPA sulphate was then found to be in wastewater at concentrations of circa 2500 ng L^{-1} (Table 5.4).

5.5.7 Confirmed structure by reference compound synthesised in vitro

Analyses of mass spectra lead to the identification of a compound previously observed after in vitro experiments (Lopardo et al. 2017). A signal at m/z 220.9681 corresponding to 4-Cl-3-methylphenol sulphate (elemental formula $C_7H_7ClO_5S$ with a mass error of 6.4 ppm), was extracted from the total ion current of each wastewater samples chromatogram (Figure 5.5) and a chromatographic peak at 5.9 min was found in all the samples analysed. Retention time was expectedly lower in the used chromatographic conditions (reverse phase) due to the metabolite higher polarity than the parent compound. BPA was in fact eluted after 7.5 min. In order to confirm that the chromatographic peak corresponds with the 4-Cl-3-methylphenol sulphate, the workflow developed by Lopardo et al., [32] was employed and mass error, and a high-resolution MS/MS spectrum (Figure 5.5) were used. A further level of confidence was added when results (retention time, mass accuracy and MS/MS fragmentation pattern) were compared to results obtained after *in vitro* studies carried out by Lopardo et al., (Lopardo et al. 2017).

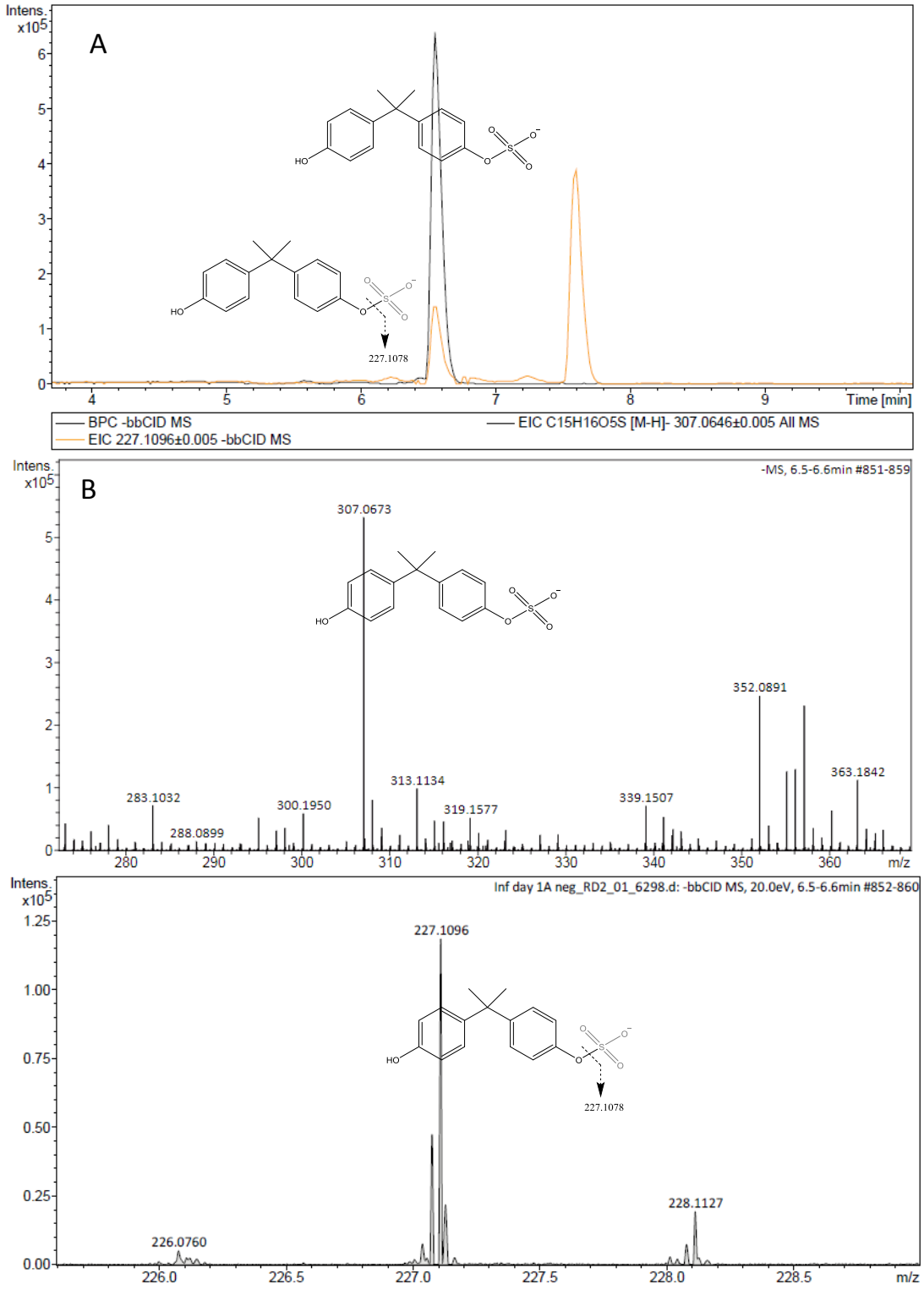


Figure 5.4 Detection and identification of BPA sulphated in wastewater by UHPLC-QTOF-MS. XICs at m/z 307.0646 and 227.1096 (0.005-Da mass-window width) (a). (b) (top) Low-energy (full-scan analysis) and (bottom) high-energy (bbCID mode) spectra and structures of the metabolite and fragment ion observed.

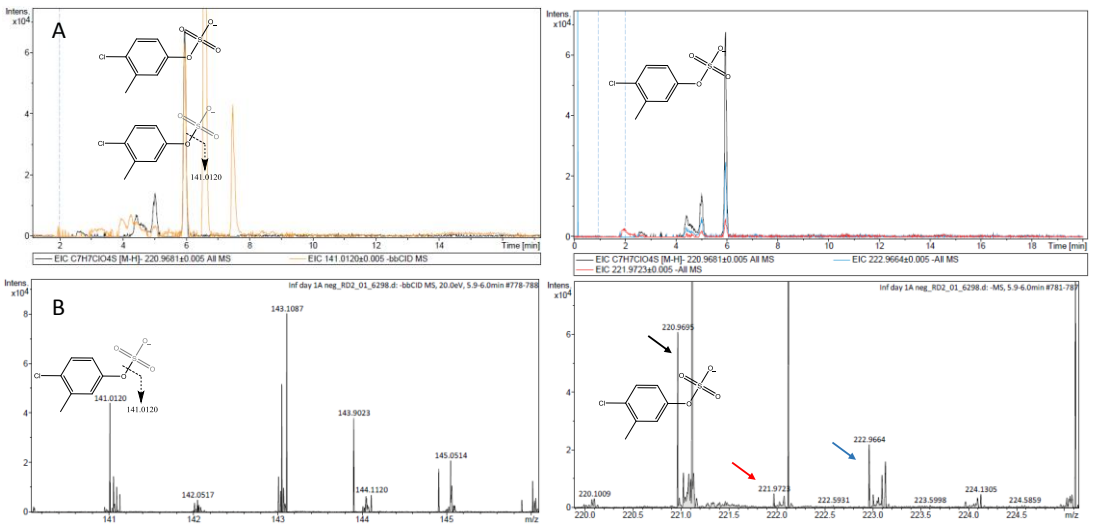


Figure 5.5 Detection and identification of 4-Cl-3-methylphenol sulphated in wastewater by UHPLC-QTOF-MS. XICs at m/z 220.9681 and 141.0113 (0.005-Da mass-window width) (a, left). XICs at m/z 220.9681, 221.9723 and 222.9664 (0.005-Da mass-window width) (a, right). (b) (left) High-energy (bbCID mode) and (right) low-energy (full-scan analysis) spectra and structures of the metabolite and fragment ion observed.

5.6 Conclusions

A new multi-residue method was developed for the analysis of 37 diverse endocrine disruptors in environmental samples. IQLs observed were < 1 µg L^{-1} for almost all compounds with some of them showing IQLs below 0.1 µg L^{-1}. MQLs achieved were < 1 ng L^{-1} for most of the compounds detected in aqueous matrices and < 1 ng g^{-1} for those detected in digested sludge. The results were similar to other studies employing different analytical techniques such as LC-TQD (Chary et al. 2012; Petrie et al. 2014, 2015; Vanderford et al. 2003). However the prospect of running on the same dataset retrospective analysis and/or untargeted screening in quest for new compounds of interest (i.e. new synthetic compounds, unknown metabolites, degradation products) makes the current method and others harnessing high resolution mass spectrometry much more versatile (Baz-Lomba et al. 2016; Boix et al. 2014; Lopardo et al. 2017; Rapp-Wright et al. 2017). Analysis of environmental samples revealed the presence of 34 out of the 37 compounds investigated. In addition, several of them were found in digested sludge, which confirms that for a more comprehensive understanding of exposure patterns and environmental impact, solid analysis cannot be neglected. Furthermore, post-acquisition data mining allowed identification and quantification in wastewater of BPA sulphate and identification of 4-Cl-3-methylphenol sulphate confirming the great potential for retrospective analysis.

5.7 Acknowledgments

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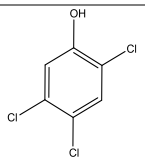
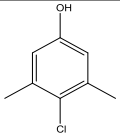
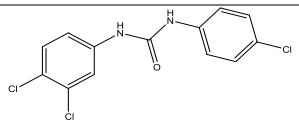
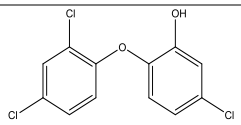
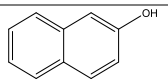
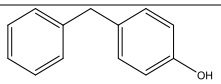
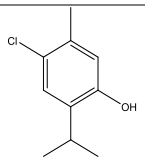
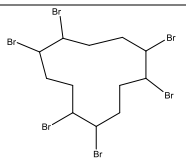
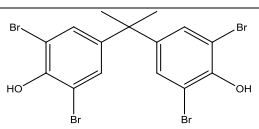
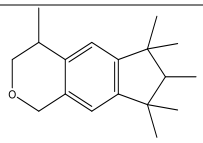
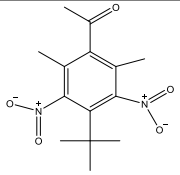
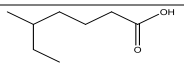
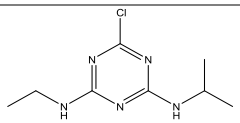
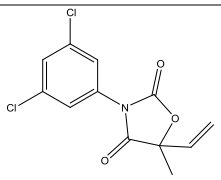
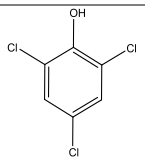
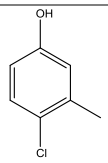
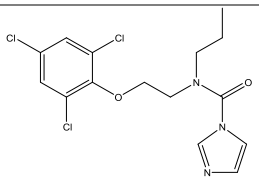
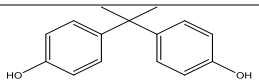
5.8 Supplementary information

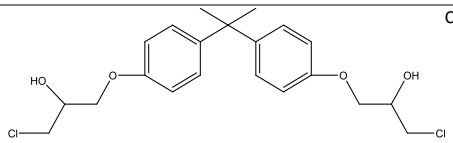
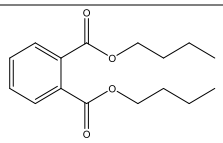
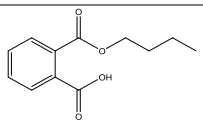
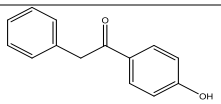
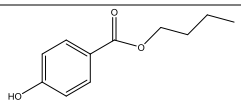
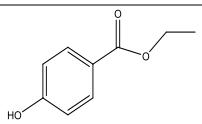
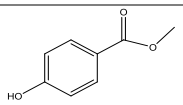
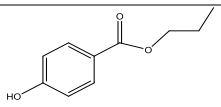
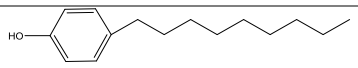
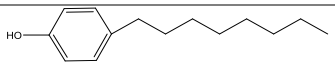
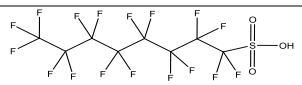
5.8.1 List of tables

5.8.2 List of figures

Table S 5.1 Endocrine disruptors selected for this study, their structures and their physical-chemical properties

Group	Analyte	Formula	Water solubility [g/L] at pH 7 Temp 25 °C	Vapor pressure (Torr, at 25 °C)	pKa	LogP	CAS number
Antimicrobial (Fungicide)	 2,4,5-trichlorophenol	$C_6H_3Cl_3O$	0,15	0,0106	7.1	3.72	95-95-4
Antimicrobial	 4-chloro-3,5-dimethylphenol	C_8H_9ClO	0,47	0,0177	9.76	3.35	88-04-0
Antimicrobial	 Triclocarban	$C_{13}H_9N_2OCl_3$	1,0E-4	6,67E-5	-0.02	6.07	101-20-2
Antimicrobial	 Triclosan	$C_{12}H_7Cl_3O_2$	1,3E-3	3,26E-5	7.8	5.34	3380-34-5
Antiseptic	 2-naphthol	$C_{10}H_8O$	0,49	1,62E-3	9.57	2.71	135-19-3
Antiseptic	 4-benzylphenol	$C_{13}H_{12}O$	0,10	1,54E-4	10.23	3.47	101-53-1
Antiseptic	 Chlorothymol	$C_{10}H_{13}ClO$	0,12	9,06E-3	10.16	4.22	89-68-9
Flame Retardant	 HBCD	$C_{12}H_{18}Br_6$	4,0E-5	7,80E-10	-	7.92	3194-55-6
Flame Retardant	 Tetrabromobisphenol A	$C_{15}H_{12}Br_4O_2$	3,8E-5	1,41E-7	8.5	9.69	79-94-7

Musk	 Galaxolide	$C_{18}H_{26}O$	0,024	4,14E-4	-	5.04	1222-05-5
Musk	 Musk ketone	$C_{14}H_{18}N_2O_5$	0,014	1,22E-5	-	2.51	81-14-1
Paint Dryer	 2-ethylhexanoic acid	$C_8H_{16}O_2$	343	0,0270	4.82	2.72	149-57-5
Pesticide	 Atrazine	$C_8H_{14}ClN_5$	0,069	1,27E-5	2.27	2.64	1912-24-9
Pesticide	 Vinclozolin	$C_{12}H_9Cl_2NO_3$	4,9E-4	1,15E-5	-3.43	3.27	50471-44-8
Pesticide (Bactericide)	 2,4,6-trichlorophenol	$C_6H_3Cl_3O$	0,32	0,0177	6.59	3.58	88-06-2
Pesticide (Bactericide)	 4-chloro-3-methylphenol	C_7H_7ClO	1,00	0,0335	9.63	2.89	59-50-7
Pesticide (Fungicide)	 Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	0,02	4,02E-10	4.84	3.98	67747-09-5
Plastic Additive	 Bisphenol A	$C_{15}H_{16}O_2$	0.071	5,34E-7	10.29	3.64	80-05-7

Plasticiser	 BADGE-2-Cl	$C_{21}H_{26}Cl_2O_4$	6,6E-3	1,56E-14	12.83	4.34	4809-35-2
Plasticiser	 dibutyl phthalate	$C_{16}H_{22}O_4$	0,025	1,08E-4	-	4.8	84-74-2
Plasticiser	 Monobutyl phthalate	$C_{12}H_{14}O_4$	478	6,40E-6	3.38	2.66	131-70-4
Preservative	 Benzylparaben	$C_{14}H_{12}O_3$	0,16	1,24E-6	8.18	3.57	94-18-8
Preservative	 Butylparaben	$C_{11}H_{14}O_3$	0,54	3,56E-4	8.22	3.41	94-26-8
Preservative	 Ethylparaben	$C_9H_{10}O_3$	2,5	7,59E-4	8.31	2.39	120-47-8
Preservative	 Methylparaben	$C_8H_8O_3$	5,6	5,55E-3	8.81	1.88	99-76-3
Preservative	 Propylparaben	$C_{10}H_{12}O_3$	1,2	9,30E-4	8.23	2.9	94-13-3
Surfactant	 4-n-nonylphenol	$C_{15}H_{24}O$	0,02	8,53E-5	10.15	6.14	104-40-5
Surfactant	 4-n-octylphenol	$C_{14}H_{22}O$	0,033	2,50E-4	10.15	5.63	1806-26-4
Surfactant	 Perfluorooctanesulfonic acid	$C_8F_{17}O_3S$	7,5	-	-3.7	7.03	1763-23-1

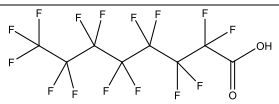
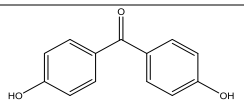
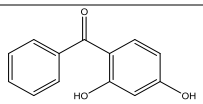
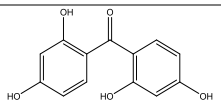
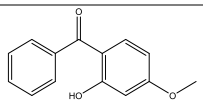
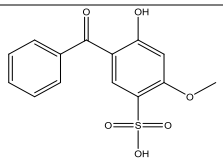
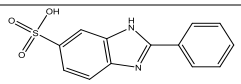
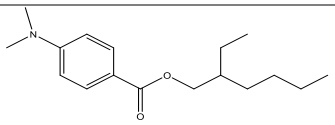
Surfactant	 Perfluorooctanoic acid	$C_8HF_{15}O_2$	13	0,274	0.5	6.44	335-67-1
UV-filter	 4,4'-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	0,73	1,58E-8	7.67	2.63	611-99-4
UV-filter	 Benzophenone-1	$C_{13}H_{10}O_3$	0,51	2,84E-7	7.72	3.15	131-56-6
UV-filter	 Benzophenone-2	$C_{13}H_{10}O_5$	3,2	6,69E-12	6.98	3.09	131-55-5
UV-filter	 Benzophenone-3	$C_{14}H_{12}O_3$	0,13	5,26E-6	7.56	4	131-57-7
UV-filter	 Benzophenone-4	$C_{14}H_{12}O_6S$	999	-	-0.7	0.99	4065-45-6
UV-filter	 Phenylbenzimidazolesulfonic acid	$C_{13}H_{10}N_2O_3S$	1000	-	-0.20	1.5	27503-81-7
UV-filter (Cosmetic)	 Padimate O	$C_{17}H_{27}NO_2$	4,7E-3	4,57E-6	2.39	6.15	21245-02-3

Table S 5.2 Mass spectrometry parameters used in the identification of endocrine disruptors including mass error in ppm in all different matrices

Analyte	**Formula**	**m/z**		**MRM transitions**		**Average mass error (ppm) (n=2)**			
		Quantifier	**Qualifier**	**Ion ratio**	**Collision energy (eV)**	**River water**	**Effluent WW**	**Influent WW**	**Digested sludge**
2,4,5-trichlorophenol	$C_6H_3Cl_3O$	194.9177	158.9419	1.1	17	2.5	1.5	5	< 1
2,4,6-trichlorophenol	$C_6H_3Cl_3O$	194.9177	158.9419	1.1	17	2.5	1.5	5	< 1
2-ethylhexanoic acid	$C_8H_{16}O_2$	143.1078	127.1128	10000	18.6	< 1	< 1	2.6	2.6

2-naphthol	$C_{10}H_8O$	143.0502	115.0556	8	20	< 1	< 1	3.5	3.5
4,4'-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	213.0557	93.0350	9	20	3.5	<1	<1	<1
4-benzylphenol	$C_{13}H_{12}O$	183.0815	106.0412	250	13	5	<1	5	<1
4-chloro-3,5-dimethylphenol	C_8H_9ClO	155.0269	119.0502	66.7	15	2	2	3.3	<1
4-chloro-3-methylphenol	C_7H_7ClO	141.0113	92.0267	1.28	14.5	<1	<1	2.8	<1
4-n-nonylphenol	$C_{15}H_{24}O$	219.1754	106.0430	14.9	17	4.5	3	1	2.5
4-n-octylphenol	$C_{14}H_{22}O$	205.1598	106.0430	3.8	17	3	1	3.5	1
Atrazine	$C_8H_{14}ClN_5$	216.1010	174.0561	0.9	17.4	4	2.5	4.5	9.5
Benzophenone-1	$C_{13}H_{10}O_3$	213.0557	135.0095	3.7	19	4	2	3.5	1.7
Benzophenone-2	$C_{13}H_{10}O_5$	245.0455	109.0301	3.5	12	2.4	2	3.6	1.2
Benzophenone-3	$C_{14}H_{12}O_3$	229.0859	151.0397	0.4	22	<1	2.6	5.6	5.6
Benzophenone-4	$C_{14}H_{12}O_6S$	307.0282	227.0721	2.5	27.4	3.3	3	2	<1
Benzylparaben	$C_{14}H_{12}O_3$	227.0714	136.0169	1	19	3.5	2.6	3.6	2
Bisphenol A	$C_{15}H_{16}O_2$	227.1078	211.0760	125	12	2.6	1	2	2.6
BADGE-2-Cl*	$C_{21}H_{26}Cl_2O_4$	411.1135	-	-	-	<1	1.5	<1	<1
Butylparaben	$C_{11}H_{14}O_3$	193.087	136.0169	2	17	3.5	2.5	3.5	2.5
Chlorothymol	$C_{10}H_{13}ClO$	183.0582	168.0348	166.7	14.5	4.4	2.7	5	<1
dibutyl phthalate	$C_{16}H_{22}O_4$	277.1445	208.0858	7.7	20	< 1	< 1	3.9	1.4
Ethylparaben	$C_9H_{10}O_3$	165.0557	136.0166	10	20	2.4	1.8	3	2.4
Galaxolide	$C_{18}H_{26}O$	259.2056	175.1126	0.5	17	< 1	1.5	3	5.4
HBCD*	$C_{12}H_{18}Br_6$	634.6436	-	-	-	2	< 1	2.3	-
MEHP	$C_{16}H_{22}O_4$	277.1445	233.1552	5.8	13.8	2.5	5	7.9	< 1
Methylparaben;	$C_8H_8O_3$	151.0401	136.0169	1.7	15	2	< 1	5	< 1
Monobutyl phthalate	$C_{12}H_{14}O_4$	221.0819	121.0300	3.3	25	2.7	2.7	2.7	< 1
Musk ketone	$C_{14}H_{18}N_2O_5$	293.1143	251.1039	6.3	26	2	2.4	5	7.9
Padimate O	$C_{17}H_{27}NO_2$	362.2115	166.0873	0.6	19	< 1	< 1	5.5	5.7
Perfluorooctanesulfonic acid	$C_8F_{17}O_3S$	498.9302	79.9580	200	31.5	3.8	5	< 1	< 1
Perfluorooctanoic acid	$C_8HF_{15}O_2$	412.9664	368.9783	0.1	11	4.3	4.3	1	< 1
Phenylbenzimidazolesulfonic acid	$C_{13}H_{10}N_2O_3S$	273.0339	193.0782	3.7	14	2.9	2.9	1.8	1.5
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	376.0381	308.0035	0.04	12.8	< 1	1.6	3.8	5.7
Propylparaben	$C_{10}H_{12}O_3$	179.0714	136.0169	3.1	15	2.8	2.2	2.2	2.2
Tetrabromobisphenol A	$C_{15}H_{12}Br_4O_2$	538.7498	288.8870	3	13.8	< 1	2.2	1.7	1.5
Triclocarban	$C_{13}H_9N_2OCl_3$	312.9708	126.0115	16	20	< 1	2.9	< 1	< 1
Triclosan*	$C_{12}H_7Cl_3O_2$	286.9439	-	-	-	< 1	1.8	< 1	1
Vinclozolin	$C_{12}H_9Cl_2NO_3$	283.9887	140.0361	41.7	14	1.4	< 1	4.2	1.4

Note: 1 for starred compounds (due to poor fragmentation) only one MRM transition could be monitored.

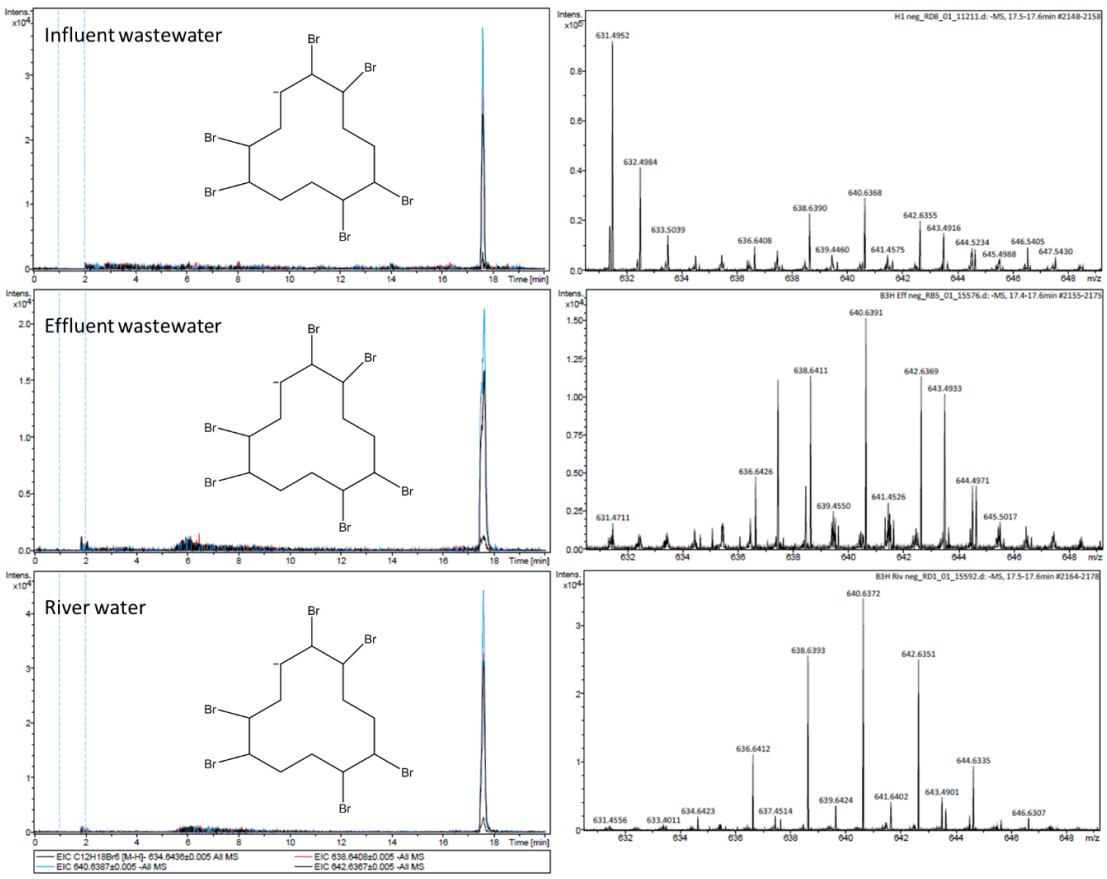


Figure S 5.1 Separation and identification HBCD in all analyzed matrices (River water, influent and effluent wastewater). XIC at m/z 634.6436 (0.005-Da mass-window width, black trace), at m/z 638.6408 (0.005-Da mass-window width, red trace), at m/z 640.6387 (0.005-Da mass-window width, blue trace) and at m/z 642.6367 (0.005-Da mass-window width, brown trace) in three different matrices (from top to bottom), and respective mass spectra of the peak eluted at 17.5 minutes.

5.9 References

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6 Conclusions and future perspectives

The present work highlighted and then filled several gaps present in literature regarding the metabolism of (and exposure to) EDCs used in personal care and consumer products. This was achieved by developing a new tool combining WBE with in vitro incubation techniques and urine analysis that enable wastewater profiling to be used for a better and more comprehensive understanding of exposure to known and newly identified potentially toxic compounds.

WBE represents an exquisite monitoring strategy that is only applicable to broadly investigated chemicals. That because a key step when undertaking these kind of studies is establishing which metabolites are going to be good biomarkers of exposure. Up to date one of the most popular method to achieve that is to perform in vitro metabolism studies. Although these kind experiments are well-established techniques to investigate the metabolism of chemical compounds, they come with huge limitation when the final goal is to identify human metabolites that are excreted in urine and detected in wastewater. For example, in vitro experiments using human subcellular fractions can provide excellent information about what type of biotransformation is likely to occur, but at the same time provide no information about excretion and detectability in wastewater which are essential in WBE. On the other hand wastewater analysis cannot serve as the only tool for the selection of good biomarkers of exposure for new chemicals too. The identification of any new potential metabolite in wastewater in fact requires confirmation that the metabolites detected are human metabolites and not due to other physical-chemical or biological agents. The newly developed tool combining wastewater profiling, in vitro metabolism studies and urine analysis overcomes such limitations permitting what was not possible to achieve with a single study before. That includes monitoring studies on known chemicals and at the same time a fast and unequivocal identification of suitable biomarkers of exposure for new chemicals. Ultimately a preliminary assessment of the exposure of populations to the new compound of interest in question can be obtained, as the study on 4-Cl-3-methylphenol proves, unveiling the great potential of the approach.

Obtaining such essential information in such a short time for new compounds along with the monitoring of the known ones permits to have a more comprehensive understanding of patterns of exposure to mixture of chemicals, which represents a real

advantage for public health decision making strategies. Some difficulties remain with the method. For example, it is lacking a tool that associates exposure to chemicals to the incidence of diseases. That can be overcome by future workers linking exposure patterns together with clinical studies that might shed light on the causes that favour the incidence of certain specific chronic diseases (e.g. diabetes, obesity, fertility problems etc.) in the studied population. Such proactive strategies could improve the overall quality of life of individuals with prevention rather than cure. Finally, prevention is also known to be more cost effective for the economy since it reduces health care costs and permits redirecting of more financial resources where needed.

6.1 Estimated spatiotemporal communitywide exposure to BPA via WBE

This chapter focusses on a literature review analysing the state of the research carried out on EDCs used in personal care and consumer products and wastewater epidemiology. In the second part a case study is reported where a WBE approach is undertaken to investigate population exposure to BPA highlighting the great potential of such approach.

Understanding the exposure pathways to emerging contaminants such as endocrine disruptors (EDCs) plays a key role for the public health monitoring. The newly emerging supra-disciplinary field of wastewater-based epidemiology (WBE), thanks to its unique approach towards retrieving epidemiological information from measuring biomarkers in wastewater, can overcome spatial and temporal limitations providing real time measurements of community-wide exposure to EDCs chemicals present in personal care products and a wider-group of chemicals that are not intended for human consumption and therefore lack comprehensive risk assessment data. A comprehensive spatiotemporal community wide study assessing the exposure to BPA was undertaken to highlight the extraordinary potential of such approach. The study was applied to a large geographical area of 2,000 km^2 and a population of ~1.5 million accounting for >75% of the overall population in the studied catchment area. For the first time the presence of BPA sulphate was monitored in wastewater and the exposure intended as intake of such chemical was evaluated using a WBA approach. Overall BPA intake was found to be below what EFSA set as the tolerable daily intake (TDI) in most locations but two WWTPs where exposure higher than the TDI was observed. Moreover temporal variations were observed in most studied WWTPs suggesting

different exposure rate throughout the week. In addition to that, the measurement of a non-consistent ratio between the amount of BPA and BPA sulphate between the different WWTPs suggests that the presence of parent compound can be due to other sources (e.g. industrial discharge and domestic direct disposal), and therefore it cannot be indicative of the exposure.

6.2 New analytical framework developed

Most of the chemicals selected for the study were prioritised based on gaps in knowledge as they are not meant for consumption. As a consequence of that, their metabolism and excretion are unknown making a selection of metabolites suitable as biomarkers of exposure rather difficult. Identifying new metabolites for scarcely investigated compounds that can also be suitable as biomarkers of exposure plays a crucial role in the context of wastewater analysis. To achieve that a new tool that consisted in a multistep workflow that combined *in vitro* techniques to urine and wastewater analysis was developed.

A semi-targeted screening was undertaken. This approach, in contrast to non-target screening that starts without any a priori information on the compounds to be identified compares masses detected in a full scan analysis against only a limited number of chemically meaningful structures that show a close relationship with the parent compound. The steps were as follows: Step 1: *In vitro* HLM/S9 assay; Step 2: *In vivo* pooled urine assay; Step 3: *In vivo* wastewater fingerprinting assay; Step 4: Analysis with HR-MSMS. This step includes metabolites identification and confirmation. The process starts with the establishment of a list of suspected metabolites harnessing ACDLab software, false positives were then excluded monitoring retention time, mass accuracy and isotope pattern and finally the structure was then confirmed by analysing the MS/MS fragmentation pattern in bbCID mode; Step 5: Data processing and Step 6: Selection of biomarkers. A further confirmation step performing a data-dependent MS/MS can be necessary for those metabolites that for different reasons do not provide an optimal MS/MS fragmentation pattern in bbCID. Four new possible metabolites were identified for the selected antimicrobial 4-Cl-3-methylphenol (PCMC) after *in vitro* HLM/S9 studies and the biotransformations observed were phase I and phase II. The comparison between the *in vitro* tests employing only HLM and those employing both HLM and S9 fraction highlighted the importance of coupling microsomes with

cytosolic enzymes to identify a more representative range of metabolites. Of the four metabolites identified only one was confirmed in the urine and wastewater samples analysed suggesting that sulphation could be the preferential metabolism pathway of the investigated antimicrobial and that PCMC sulphated would be the only metabolite excreted. Furthermore, the presence of the metabolite in the analysed matrices indicates human internal exposure to the antimicrobial despite it being utilised in products meant for external use.

6.3 New biomarkers identified

In this chapter newly developed assays combining wastewater analysis, urine analysis and in vitro incubation techniques were applied to compounds with an unknown metabolism with the scope to identify metabolites produced in vivo, excreted in urine and present in wastewater, and can therefore represent suitable biomarkers of exposure for future studies.

Similarly, to the previous study phase I and II biotransformations were observed. Five of the newly discovered metabolites (HO-Met1, OC-Met1, 4-BenzPh-Met4, 4-BenzPh-Met5 and 4-BenzPh-Met6) and one previously known metabolite (BPA-Met3) were then detected in urine and/or wastewater. The difference in number and type of compounds and metabolites observed in the two different samples was expected since the two different samples (urine and wastewater) were collected in two different times and locations representing two different populations that could have been exposed to different chemicals. However, consistency was observed in detecting metabolites of high usage UV filters. The findings confirm a significant potential for this new approach in particular in its future application in verification of public exposure to chemicals via WBE.

6.4 New quantitative method and EDCs quantified at a catchment scale

A new analytical LC-MS method to perform the a quantitative analysis of all the selected compounds of interests (chemically diverse EDCs) and respective metabolites combined with an untargeted screening for more compounds of interest (in solid and liquid matrices) was needed to allow future monitoring studies, identification of new biomarkers of exposure and to perform retrospective analysis.

Finally, a new analytical method for a comprehensive investigation of 37 EDCs and newly identified biomarkers of exposure was developed. For the first time very diverse chemical compounds ranging from flame retardants to fragrances and including UV filters, plasticizers, polyfluorinated substances, surfactants and antimicrobials were analysed with one multiresidue method. Through this method, the prevalence of 37 compounds was studied in influent and effluent wastewater samples, river water upstream and downstream to the WWTPs as well as in digested sludge. The significative presence of 60% of the analysed compounds in digested sludge confirmed that solid analysis is crucial for a comprehensive environmental risk assessment and for a correct evaluation of biomarkers loads in order to have an accurate estimate of internal exposure to parent compounds. Furthermore, the selection of high resolution mass spectrometry (HRMS) coupled with liquid chromatography (LC) provided the possibility of identifying compounds which were not included in the initial analysis (post-target or retrospective analysis) that can be achieved without the need for producing extra samples, on raw data that can be stored indeterminately. This possibility permitted the investigation of newly identified compounds or chemicals that were not yet integrated into the monitoring strategies currently in use such as compound's metabolites (e.g. BPA sulphate and 4-Cl-3-methylphenol sulphate). Metabolites were identified on different levels of confidence and eventually quantified so that a WBE approach could be undertaken. The results obtained in this present work lead to the conclusion that the impact of the exposure to EDCs and other chemicals not intended for human consumption might need to be reconsidered and their impact on the aquatic ecosystem along with their metabolites should be also monitored to verify their potential environmental impact.

6.5 Future work

Mechanisms of transformation of EDCs in wastewater and the environment are largely unknown therefore stability and mechanisms of transformation of EDCs and their metabolites in wastewater and the environment should be investigated undertaking microcosm experiments. Such studies are needed to verify the stability and usefulness of chosen biomarkers as well as to evaluate all exposure routes and risks. For newly discovered metabolites standards should be synthesised to permit a full quantification and *in vivo* metabolism studies should be performed to understand excretion ratio and apply a WBE approach (estimate exposure to given chemicals by measuring

biomarkers in wastewater). Finally, the validated method should be used to support monitoring studies to provide a greater understanding of the presence, fate, and ecological impact of EDCs in wastewaters and the environment in a vast catchment area in the south west of England with the ultimate goal of increasing knowledge of the effects of the exposure to these compounds on the whole populations. Furthermore the new analytical workflow represents a powerful tool that can change exposure studies coupling real-time monitoring with screenings performed with different degrees of selectivity for new compounds of interest that can improve human biomonitoring. In addition to that, thanks to the possibility of retrospective analysis, exposure to more compounds of interest can be investigated without more samples needed enabling a more comprehensive analysis of the relationship between exposure and effects. Ultimately the new analytical tool will permit proactive rather than reactive strategies.

To further confirm WBE as a useful method in exposure assessments more studies will be performed. Sampling campaigns will be designed to asses more in detail spatial and temporal variability within communities. Once a large and comprehensive database is crated data mining and analytical modelling will be used to identify patterns that could help explaining and predicting the incidence of certain diseases. One limitation to such approach is the inability to understand exposure pathways as excretion of biomarkers depends on the overall intake regardless of the source. To overcome this limitation *in vivo* studies will be performed to investigate absorption, distribution, and excretion of the selected chemicals. A better understanding of absorption and distribution would provide for example more information about potential sources of exposure and potential for bioaccumulation. A better understanding of excretion rates would guarantee instead smaller uncertainty when back calculating intakes.

Finally studies will be necessary to establish whether or not, at the assessed level of internal exposure to one or many compounds simultaneously, there is potential for pharmacological/toxicological effect on humans and then compare with epidemiological data.

7 PhD activities

7.1 Publications

1. Lopardo, L., Petrie, B., Proctor K., Youdan J., Barden, R., Kasprzyk-Hordern, B., Water fingerprinting for community-wide exposure assessment triclosan, Environmental Science and Technology, 2018, in preparation (tentative title)

2. Lopardo, L., Petrie, B., Camacho-Muñoz, D., Macias, D., Proctor, K., Rice, J., Rydevik, A., Youdan, J., Barden, R., ,Kasprzyk-Hordern, B., Endocrine disruptors in the environment, Water Research, 2018, in preparation (tentative title)

3. Lopardo, L., Petrie, B., Proctor K., Youdan J., Barden, R., Kasprzyk-Hordern, B., Water fingerprinting for community-wide exposure assessment to endocrine disrupting chemicals in household products: exposure to bisphenol A, Environmental Health Perspectives, 2018, submitted for publication

4. Petrie, B., Lopardo, L., Proctor, K., Youdan, J., Barden, R., Kasprzyk-Hordern, B., Impact of industrial wastewater discharges on bisphenol-A levels in municipal wastewaters and the receiving environment, Environmental Pollution, 2018, submitted for publication

5. Lopardo, L., Adams, D., Cummins, A., Kasprzyk-Hordern, B., Verifying community-wide exposure to endocrine disruptors in personal care products – In quest for metabolic biomarkers of exposure via in vitro studies and wastewater-based epidemiology, (2018) Water Research, 143, pp. 117-126.

6. Rice, J., Proctor, K., Lopardo, L, Evans S, Kasprzyk-Hordern, B, 2018, Stereochemistry of ephedrine and its environmental significance: Exposure and effects directed approach, Journal of Hazardous Materials 348 (2018) 39–46

7. Gracia-Lor, E., Castiglioni, S., Bade, R., Been, F., Castrignanò, E., Covaci, A., González-Mariño, I., Hapeshi, E., Kasprzyk-Hordern, B., Kinyua, J., Lai, F. Y., Letzel, T., Lopardo, L., Meyer, M. R., O'Brien, J., Ramin, P., Rousis, N. I., Rydevik, A., Ryu, Y., Santos, M. M., Senta, I., Thomaidis, N. S., Veloutsou, S., Yang, Z., Zuccato, E. and Bijlsma, L., 2017. Measuring biomarkers in wastewater as a new source of epidemiological information:Current state and future perspectives. Environment International, 99, pp. 131-150.

8. Lopardo, L., Cummins, A., Rydevik, A. and Kasprzyk-Hordern, B., 2017. New Analytical Framework for Verification of Biomarkers of Exposure to Chemicals Combining Human Biomonitoring and Water Fingerprinting. Analytical Chemistry, 89 (13), pp. 7232-7239.

9. Lopardo, L., Rydevik, A. and Kasprzyk-Hordern, B., 2017. A new analytical framework for multi-residue analysis of chemically diverse endocrine disruptors in complex environmental matrices utilising ultra-performance liquid chromatography coupled with high resolution tandem quadrupole time-of-flight mass spectrometry. Analytical and Bioanalytical Chemistry, submitted for publication

7.2 Oral Presentations

- Lopardo, L., Community-wide exposure routes and risks from environmental endocrine disrupting chemicals, Department of Chemistry Bolland Symposium, University of Bath, 5th June 2017

- Lopardo, L., Cummins, A., Rydevik, A., Kasprzyk-Hordern, B., Combining WBE with in vitro studies: a new approach for population wide exposure assessments to EDCs, Garning/Munich, Germany, 2nd of November 2016

- Lopardo, L., Cummins, A., Rydevik, A., Kasprzyk-Hordern, B., A new analytical strategy to evaluate community-wide exposure to endocrine disrupting chemicals in personal care products, Testing the Waters 2017, Lisbon, Portugal

Appendix A: List of reports

Report 1 Detection and identification of PCMC metabolites by UHPLC-QTOF-MS following *in-vitro* HLM assay.

Sample Name 4-Cl-3-Me_1_neg and PCMC_10ul_enz_B_Neg_2 XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC and relative isotopes following *in-vitro* HLM assay for verification of metabolic profile of PCMC.

Sample Name 4-Cl-3-Me_2_neg and PCMC_10ul_enz_B_Neg_2 XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC and relative isotopes following *in-vitro* HLM assay for verification of metabolic profile of PCMC (duplicate sample)

Sample Name 4-Cl-3-Me_Blank_neg and PCMC_blank_Neg XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC and relative isotopes following *in-vitro* HLM assay for verification of metabolic profile of PCMC (blank control)

Report 2 Detection and identification of PCMC metabolites by UHPLC-QTOF-MS following *in-vitro* HLM/S9 assay.

Sample Name S9_4-Cl-3-Me_A_6_Hour_Neg and 4_Cl_6hA_Neg XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC sulfated, PCMC sulfated and hydroxylated, PCMC and relative isotopes (including bbCID fragmentation pattern for phase II metabolites), following *in-vitro* HLM/S9 assay (6 hour sampling point) for verification of metabolic profile of PCMC.

Sample Name S9_4-Cl-3-Me_B_6_Hour_Neg and 4_Cl_6hB_Neg XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC sulfated, PCMC sulfated and hydroxylated, PCMC and relative isotopes (including bbCID fragmentation pattern for phase II metabolites), following *in-vitro* HLM/S9 assay (6 hour sampling point) for verification of metabolic profile of PCMC. (duplicate sample)

Sample Name S9_4-Cl-3-Me_Blank_6_Hour_Neg and 4_Cl_6hBlank_Neg XIC and mass spectrum of PCMC hydroxylated, PCMC glucuronidated, PCMC sulfated, PCMC sulfated and hydroxylated, PCMC and relative isotopes (including bbCID fragmentation pattern for phase II metabolites), following *in-vitro* HLM/S9 assay (6 hour sampling point) for verification of metabolic profile of PCMC. (blank control)

Report 3 Detection and identification of PCMC metabolite by UHPLC-QTOF-MS following urine analysis.

Sample Name Urine_141_A neg XIC and mass spectrum of PCMC sulfated (including bbCID fragmentation pattern) and relative isotopes, following direct *in-vivo* urine profiling assay.

Sample Name Urine_141_B neg XIC and mass spectrum of PCMC sulfated (including bbCID fragmentation pattern) and relative isotopes, following direct *in-vivo* urine profiling assay.

Report 4 Detection and identification of PCMC metabolites by UHPLC-QTOF-MS following wastewater analysis.

Sample Name Inf day 1A neg XIC and mass spectrum of PCMC and PCMC sulphated (including bbCID fragmentation pattern) and relative isotopes.

Report 5 MRM fragmentation pattern of PCMC standard solution.

Sample Name MRM_4Cl3MPox_Met2_STD_5 MRM fragmentation pattern of PCMC standard solution

Display Report

Analysis Info

Acquisition Date 6/18/2015 3:59:20 PM

Sample Name 4-Cl-3-Me_1_neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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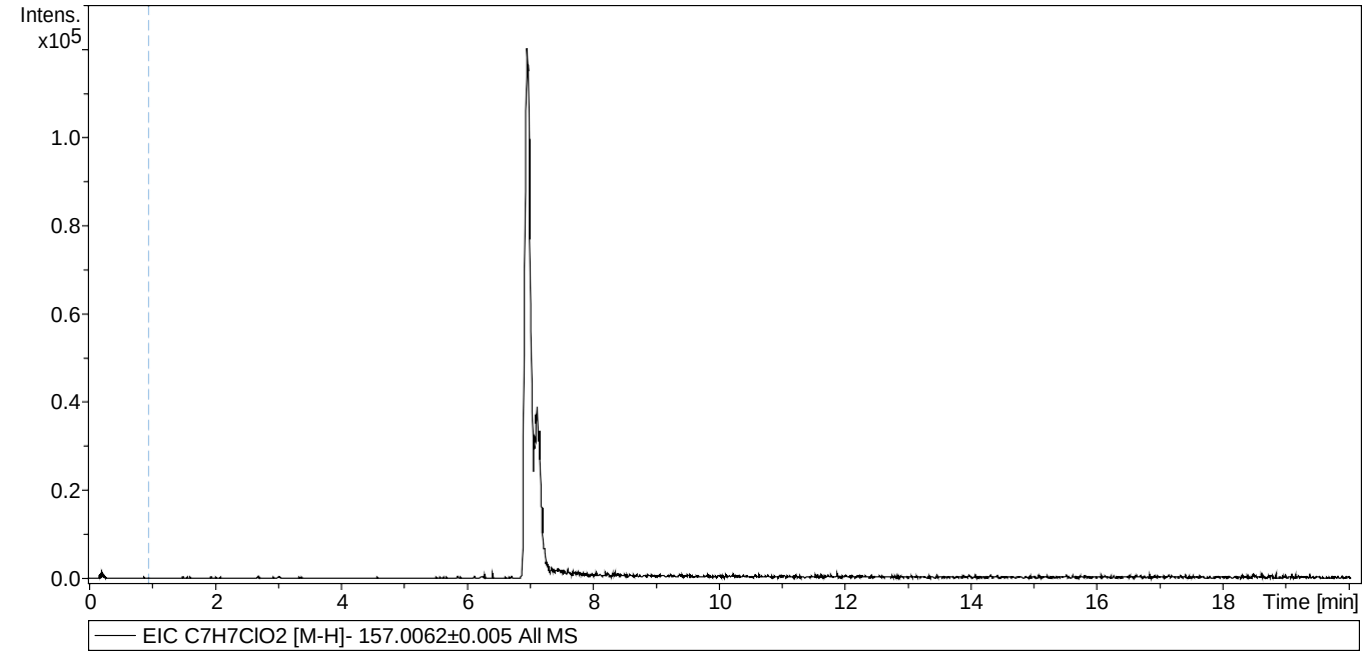
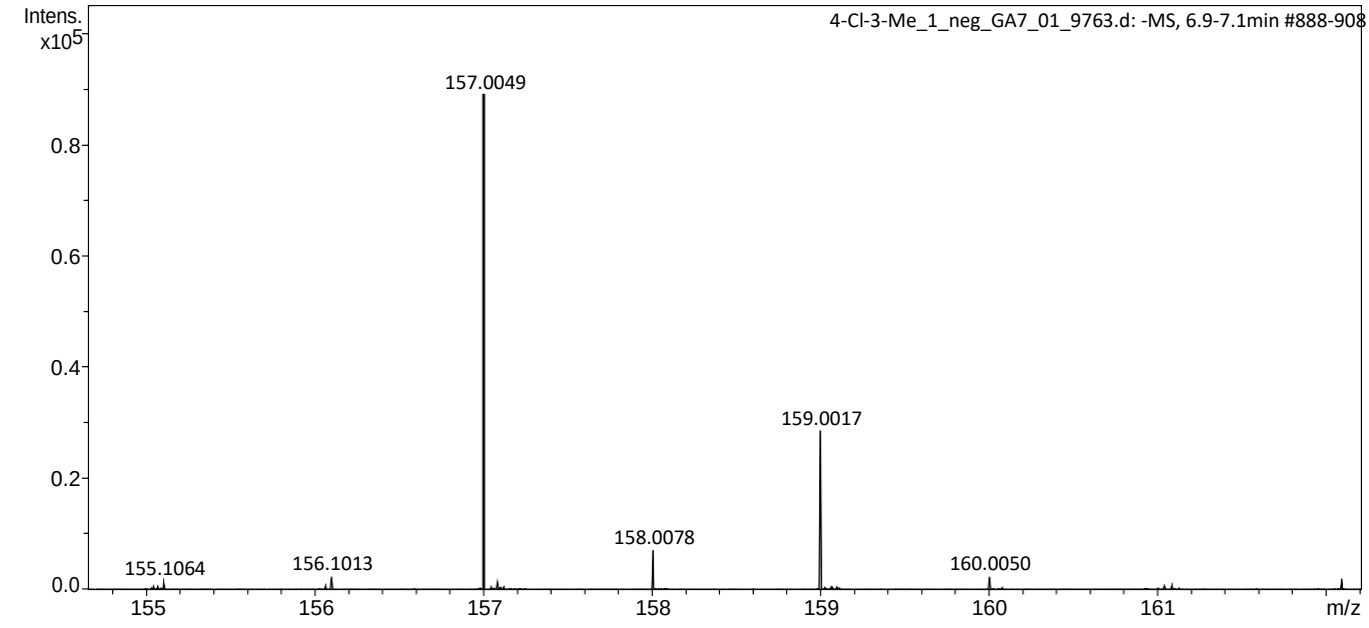
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Display Report

Analysis Info

Acquisition Date 6/18/2015 3:59:20 PM

Sample Name 4-Cl-3-Me_1_neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

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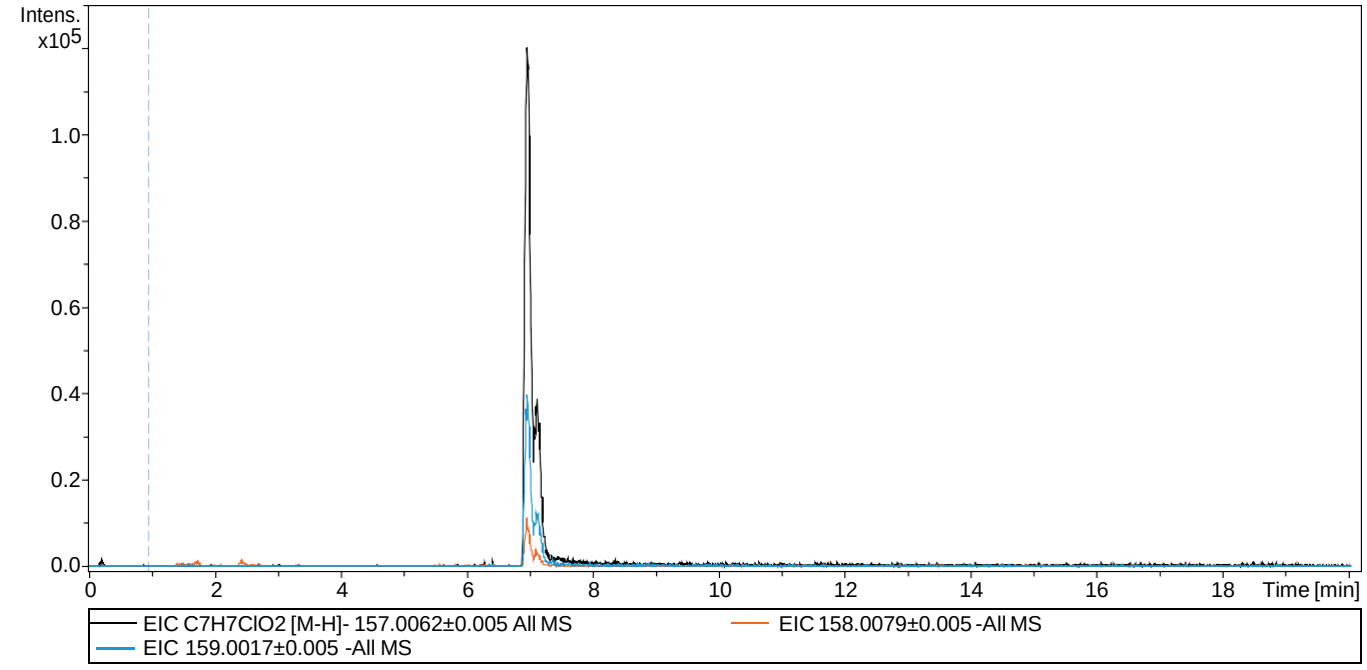

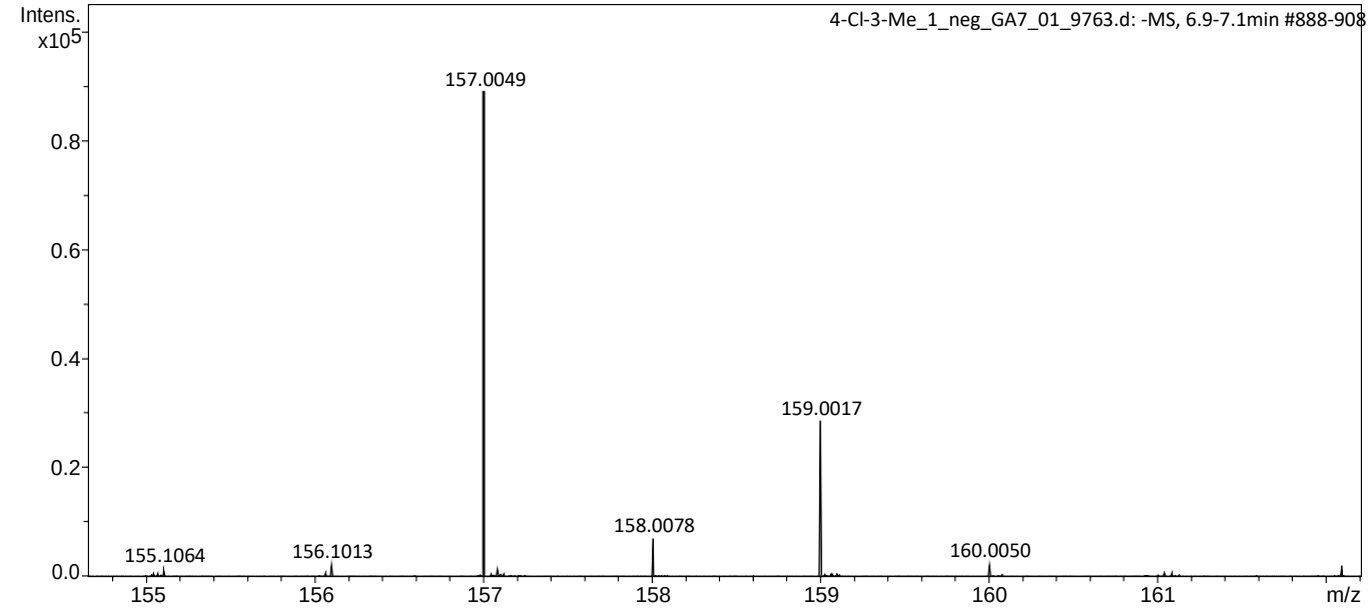

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Display Report

Analysis Info

Acquisition Date 11/11/2016 01:26:22

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

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Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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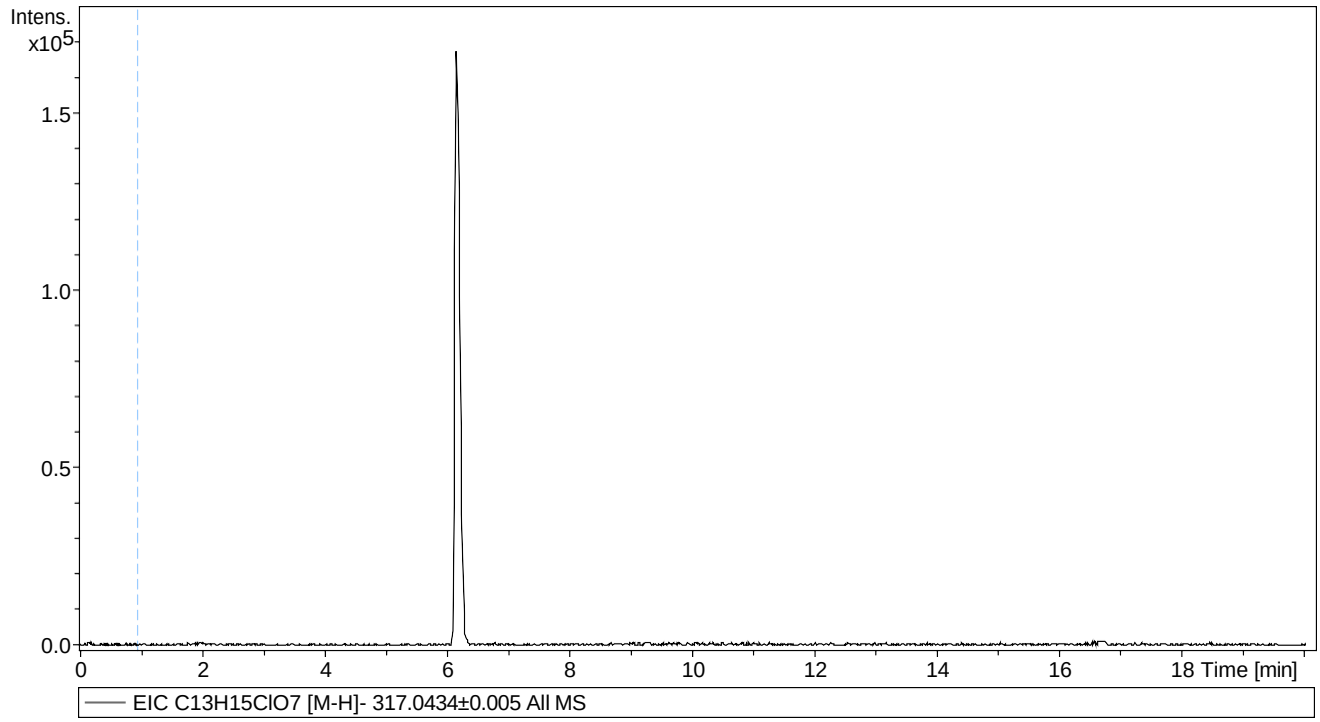

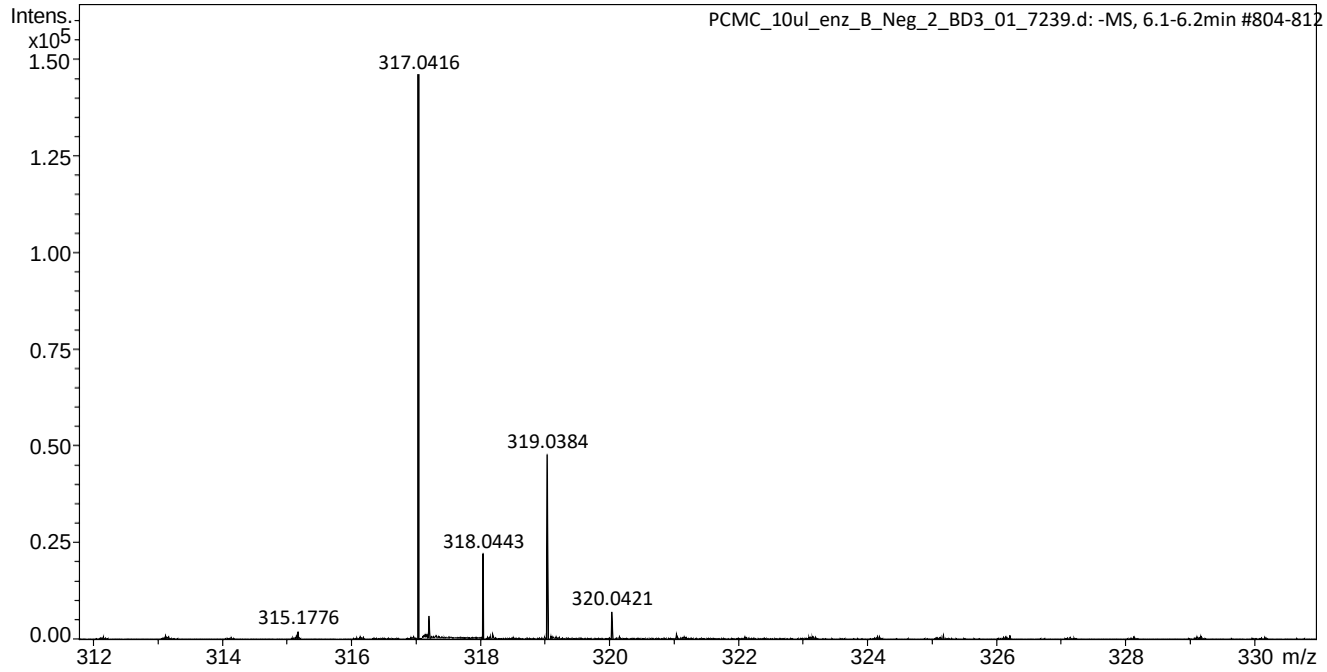

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Analysis Info

Acquisition Date 11/11/2016 01:26:22

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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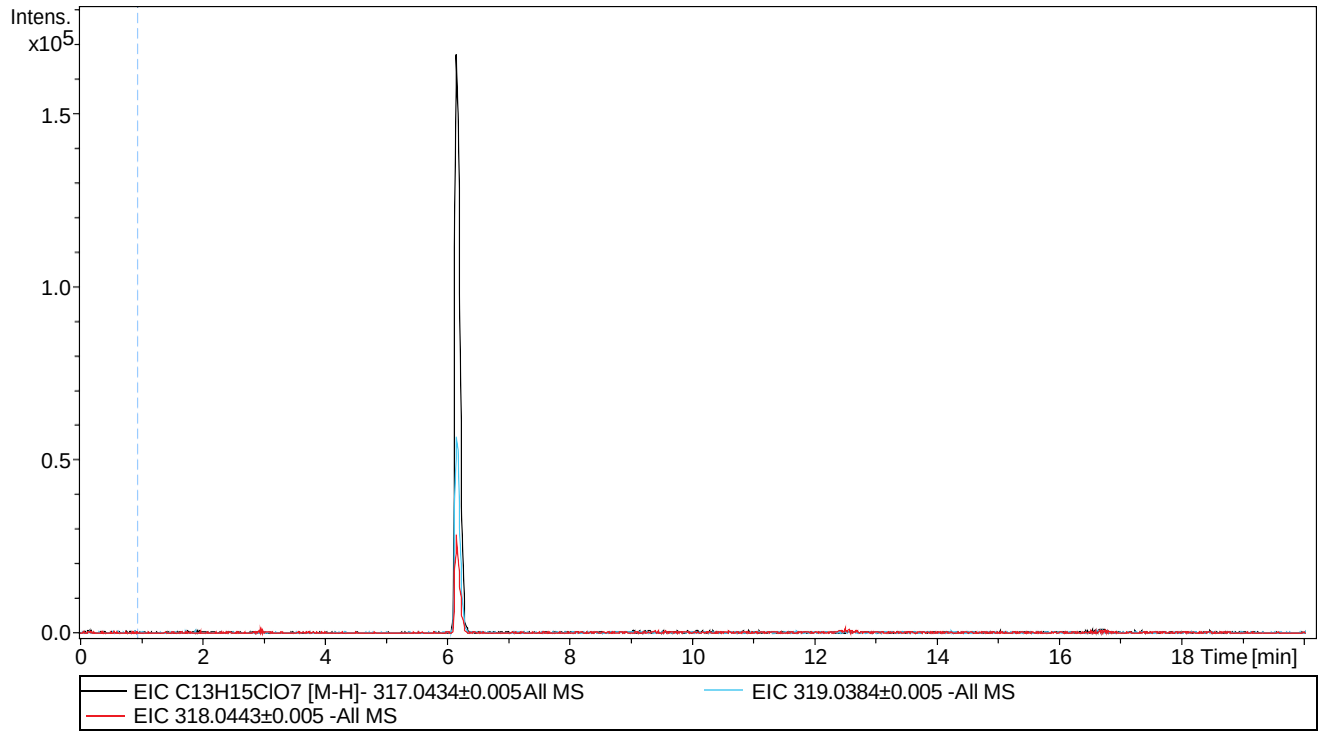

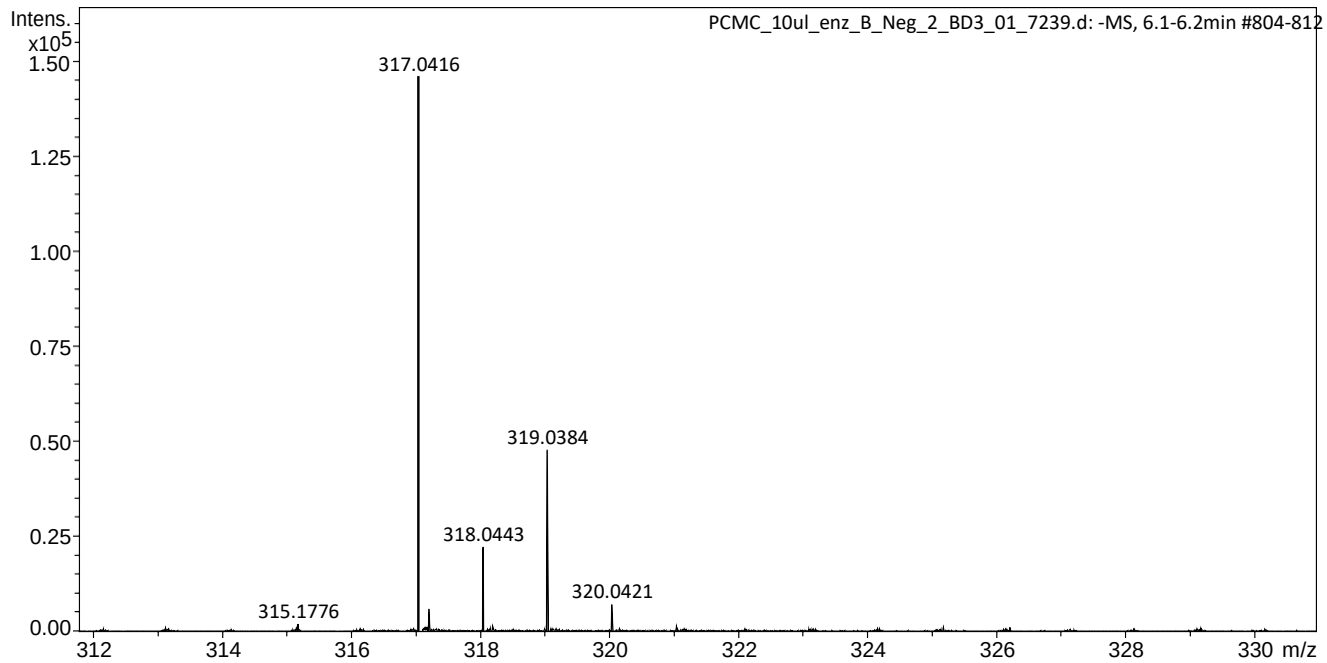

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Analysis Info

Acquisition Date 11/11/2016 01:26:22

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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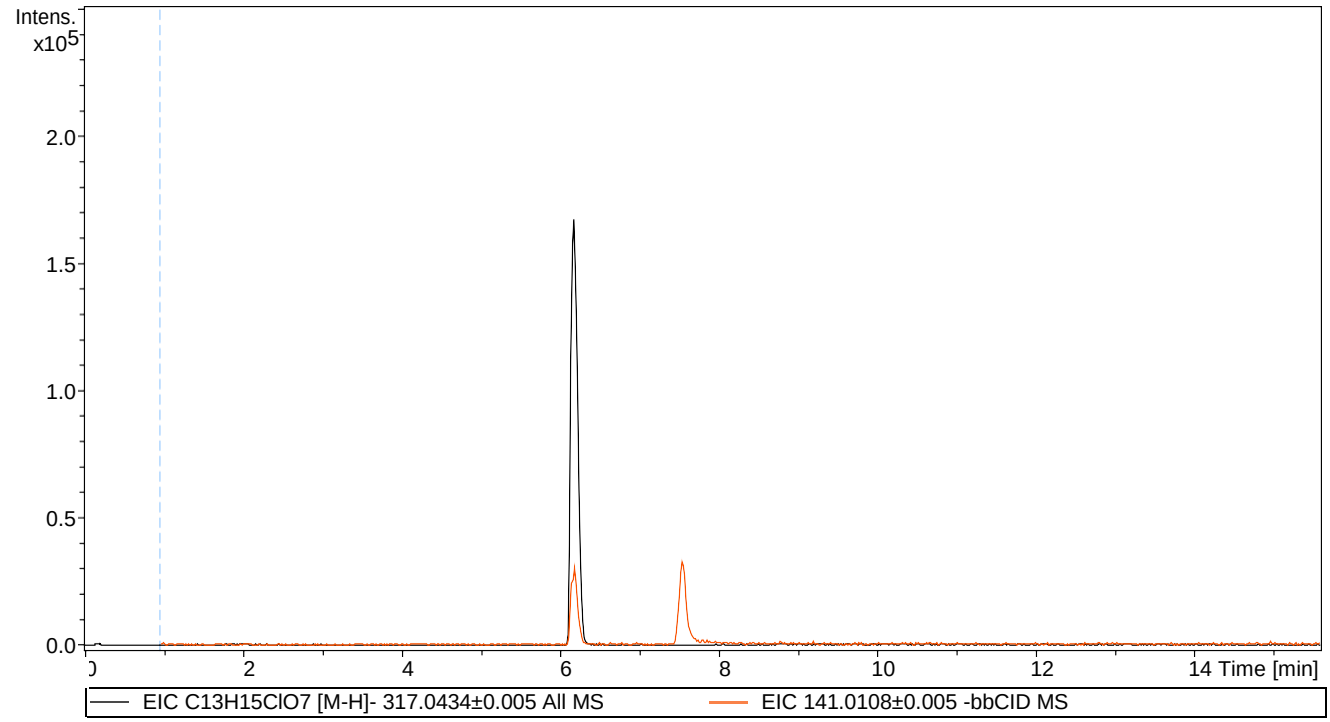

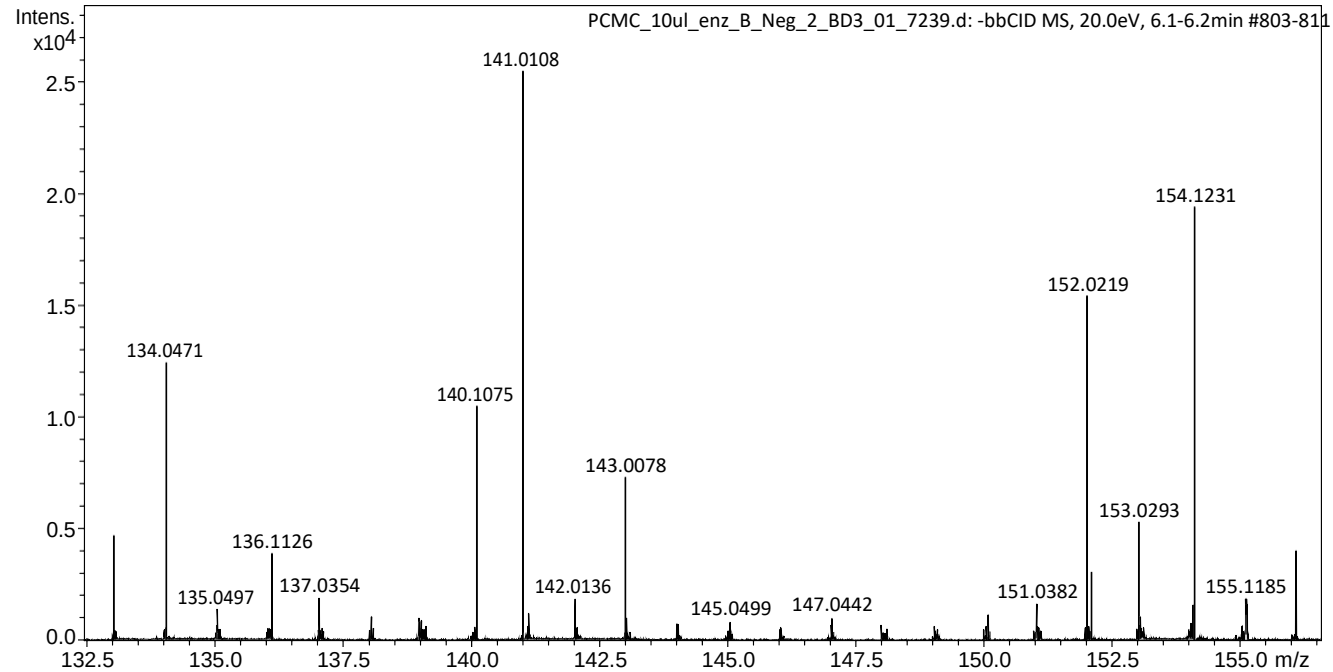

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Analysis Info

Acquisition Date 6/18/2015 6:27:59 PM

Sample Name 4-Cl-3-Me_2_neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
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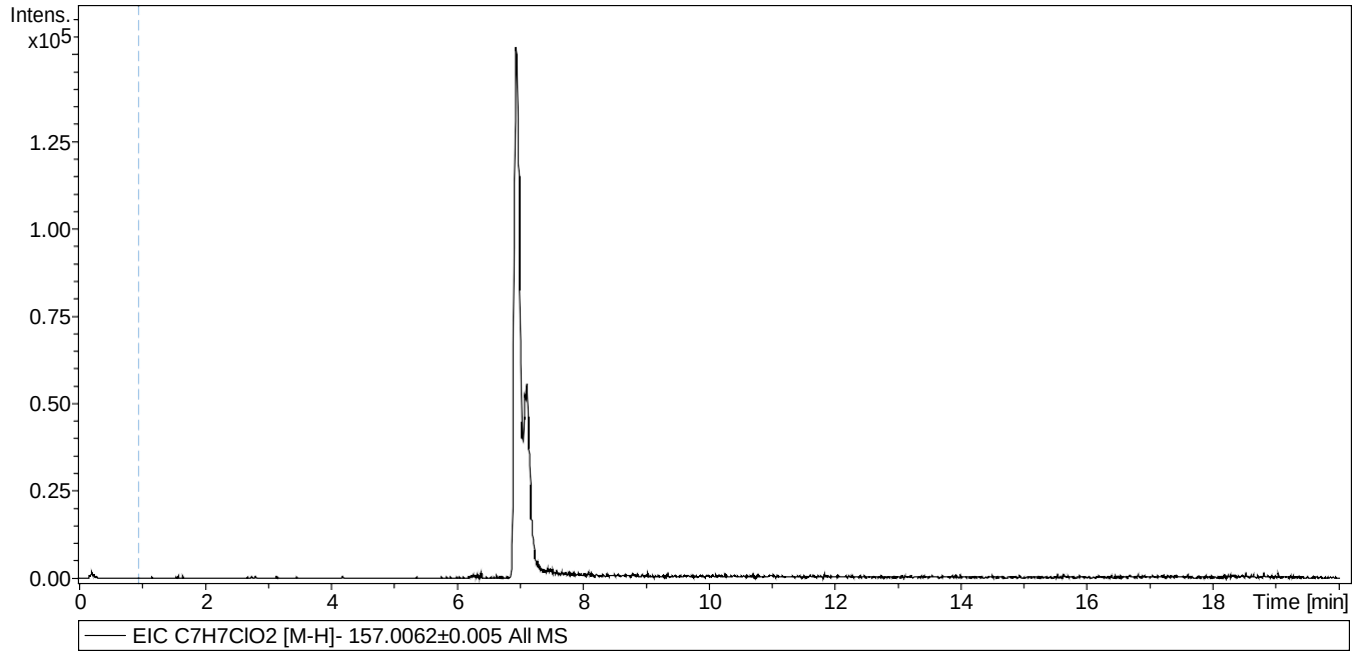

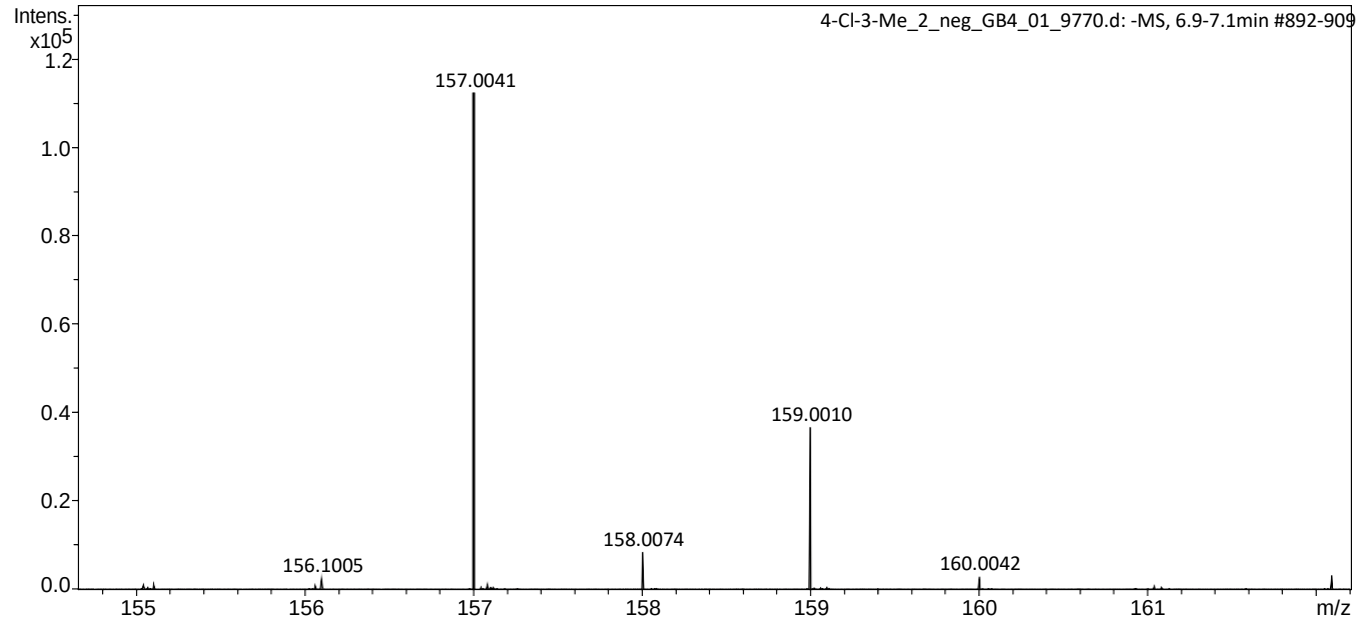

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Analysis Info

Acquisition Date	6/18/2015 6:27:59 PM		
Sample Name	4-Cl-3-Me_2_neg	Operator	CCAF
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

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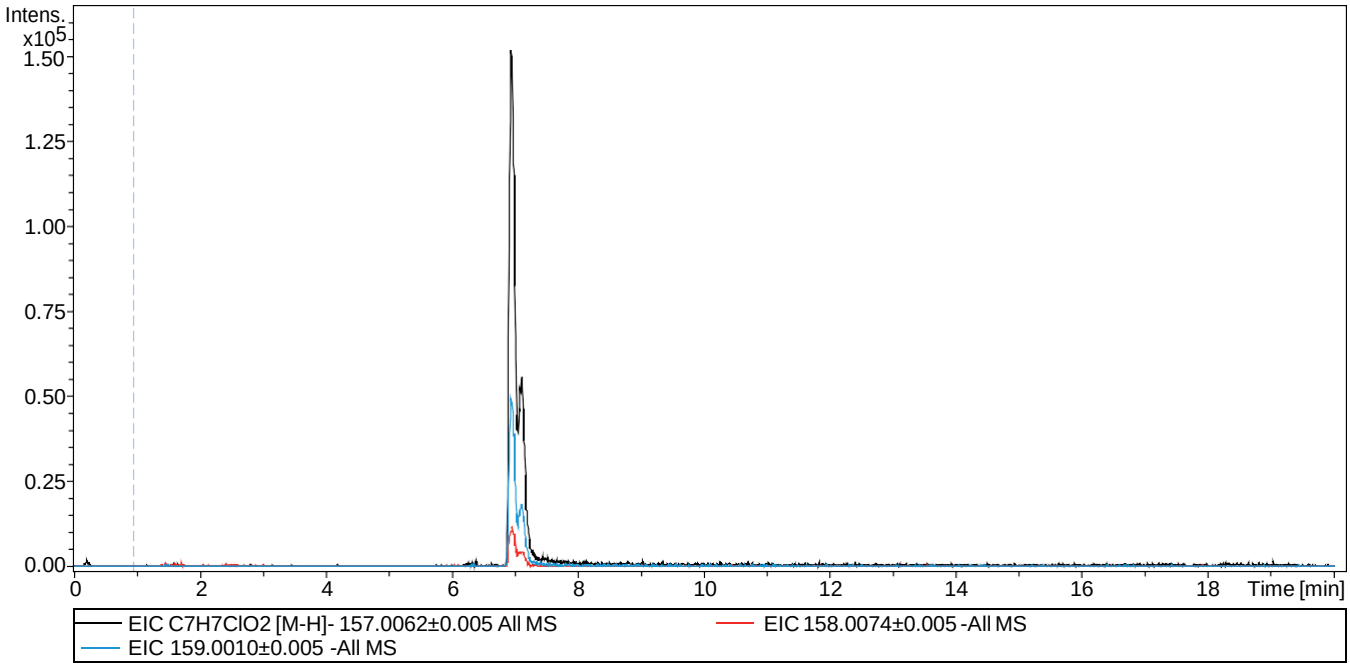

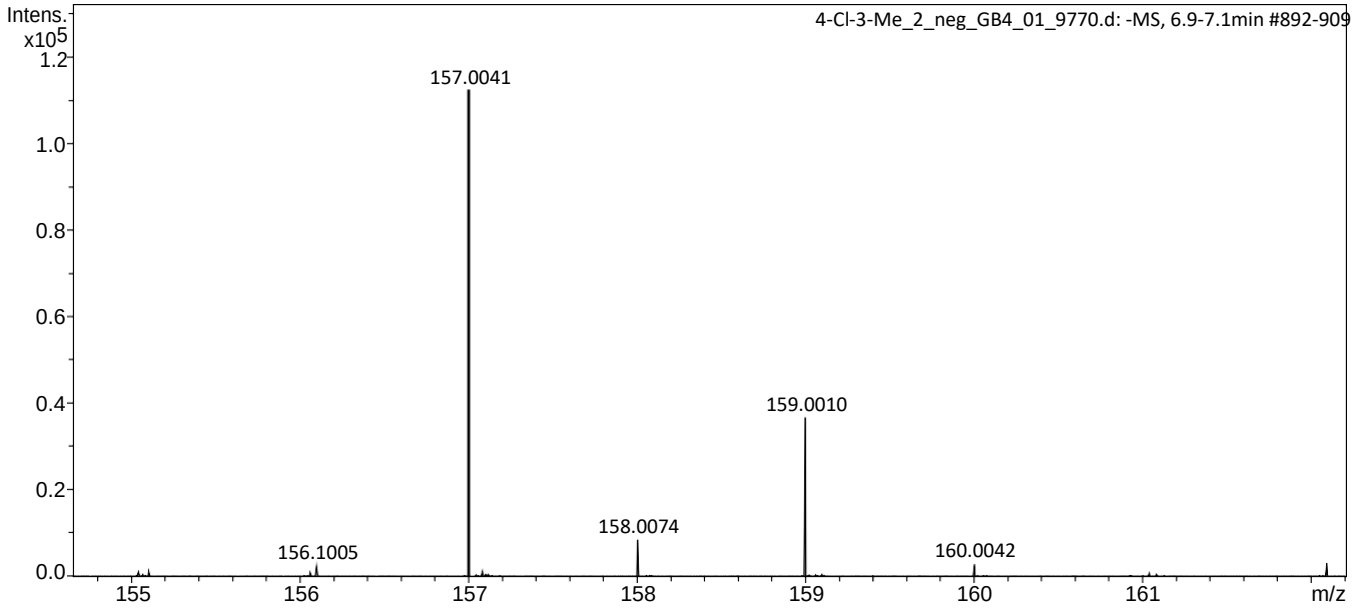

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Display Report

Analysis Info

Acquisition Date 11/11/2016 01:05:06

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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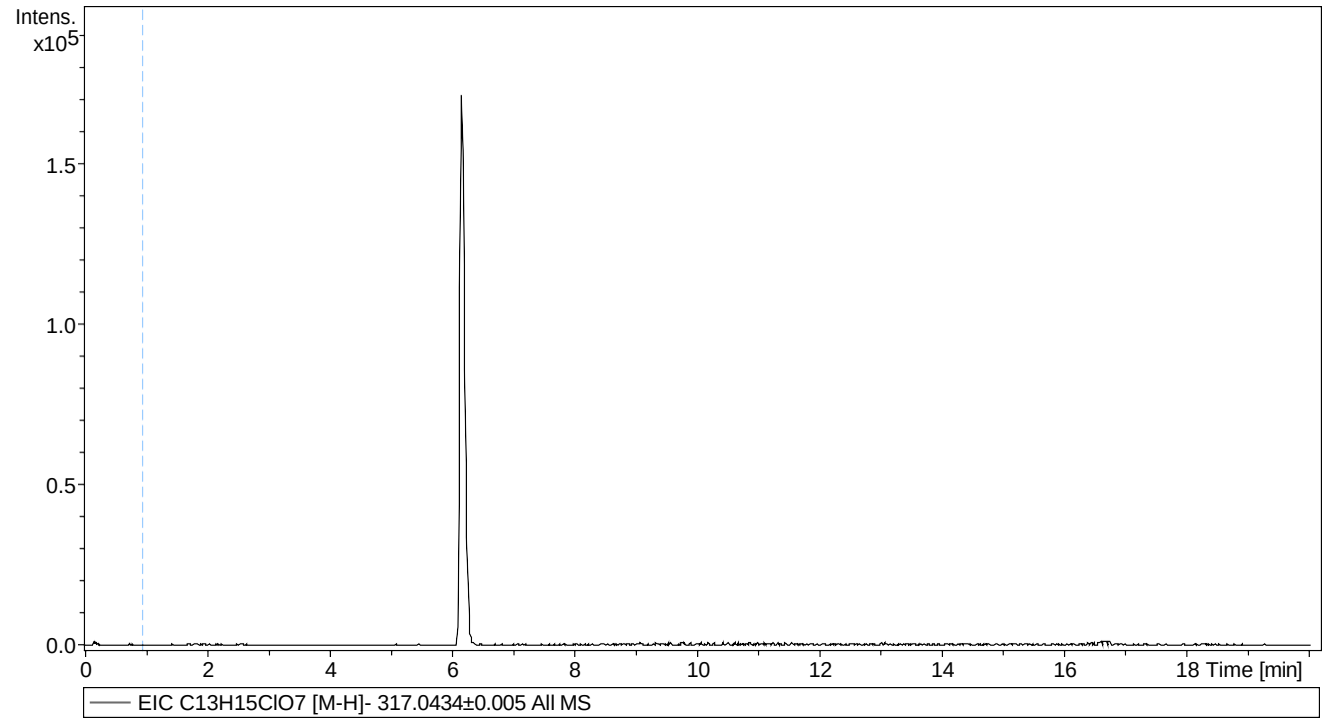

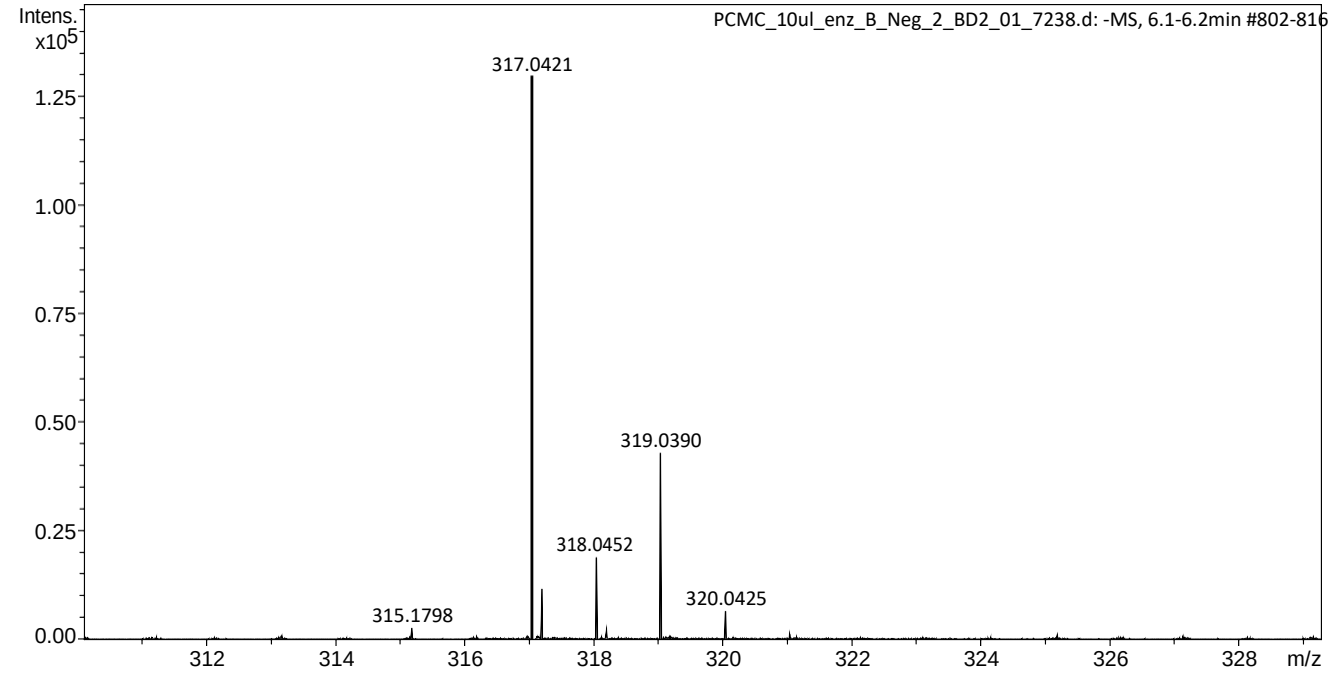

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Analysis Info

Acquisition Date 11/11/2016 01:05:06

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

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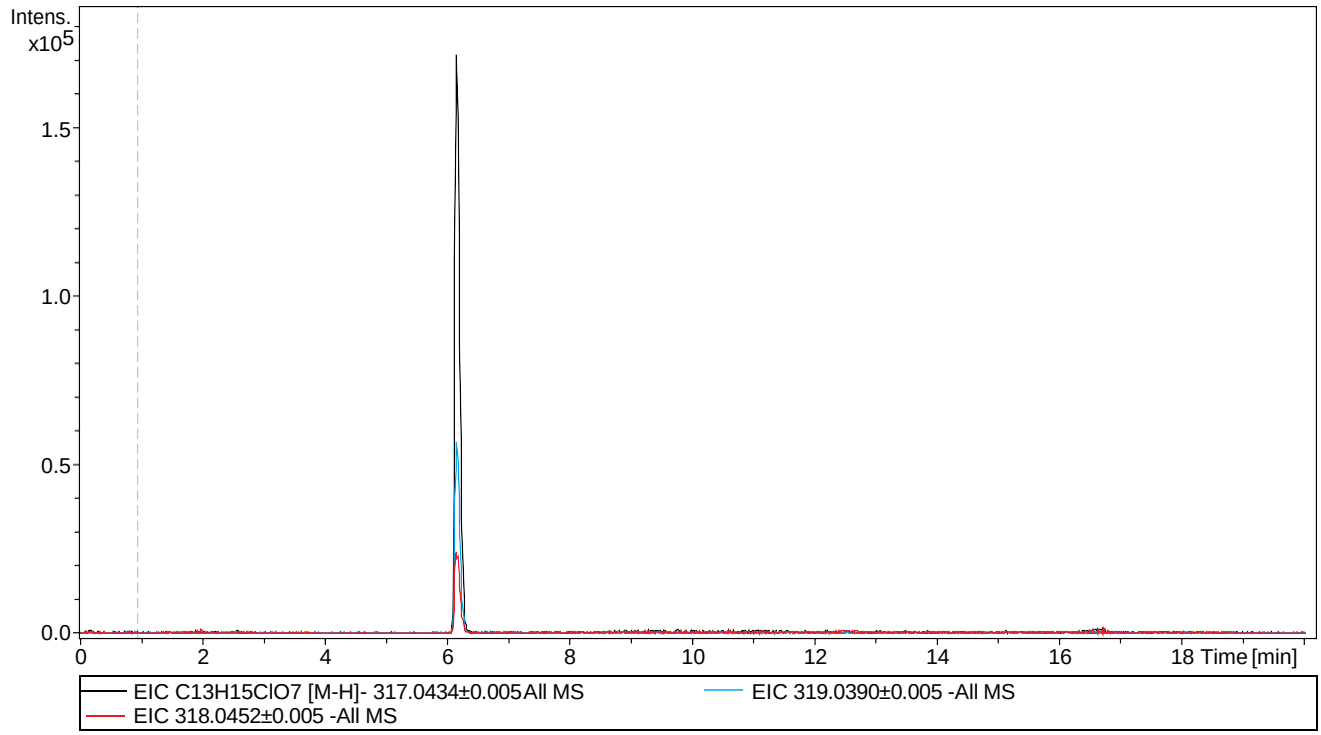

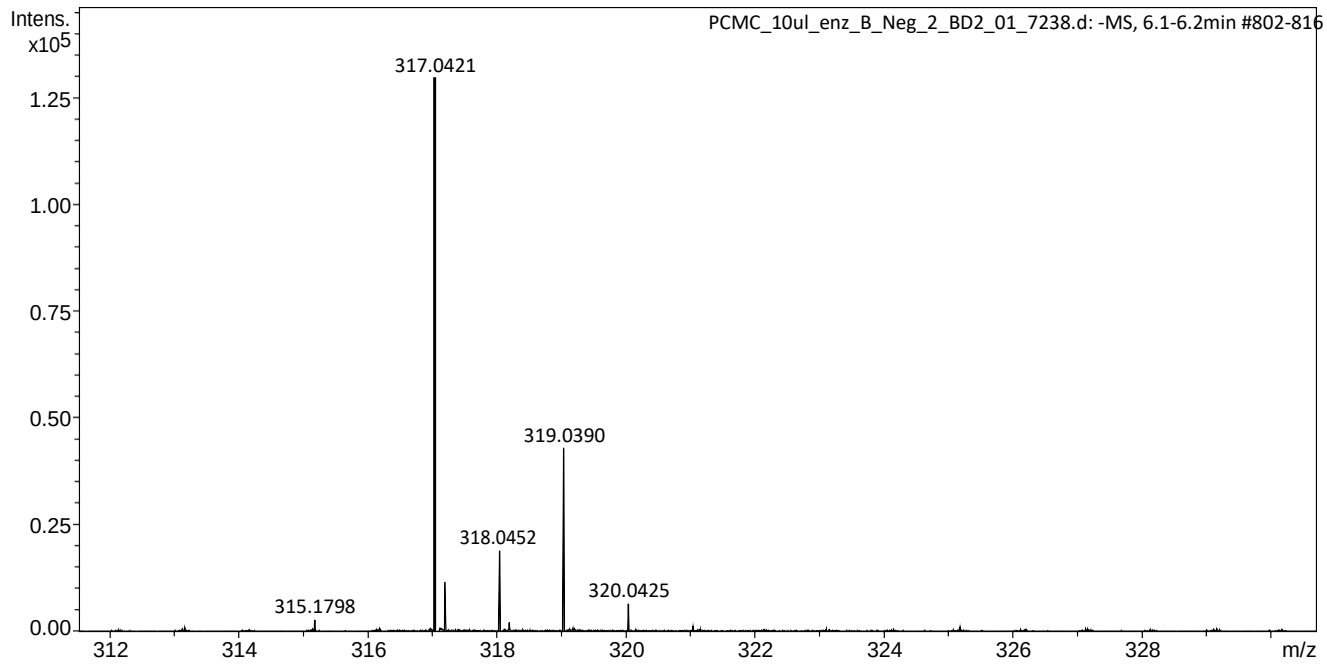

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Analysis Info

Acquisition Date 11/11/2016 01:05:06

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

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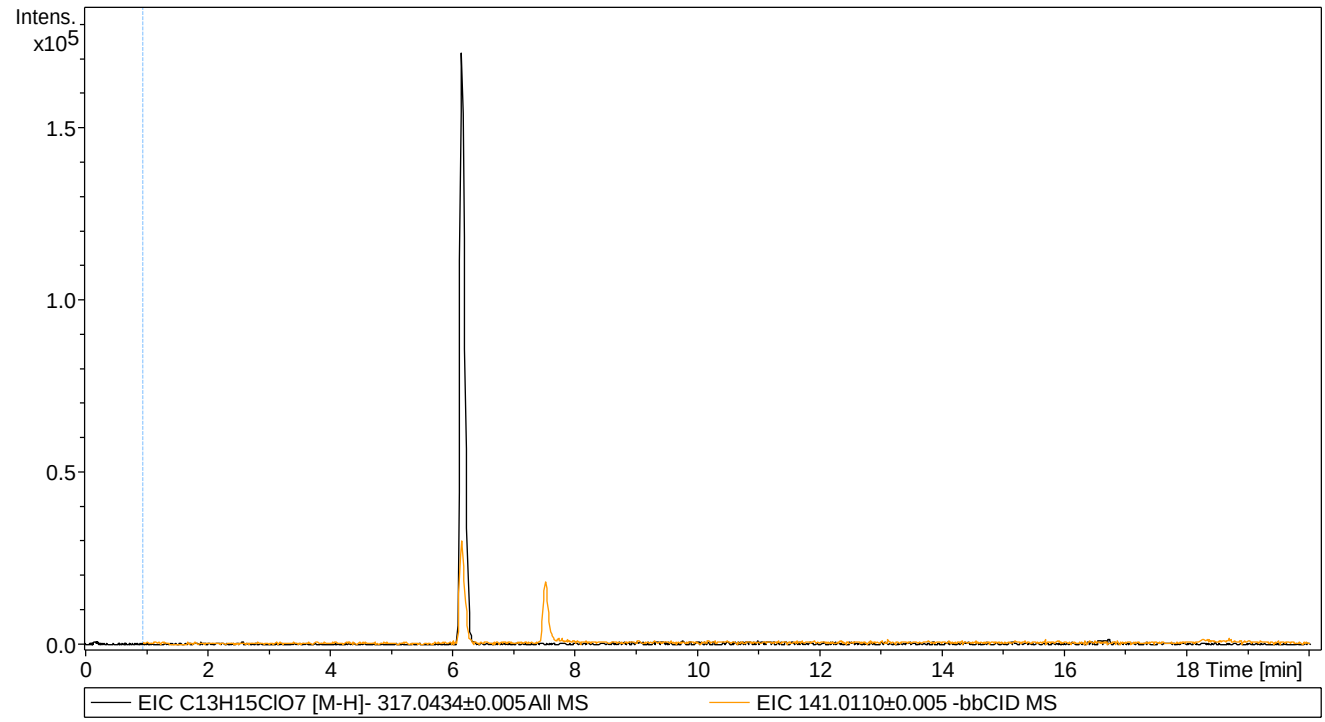

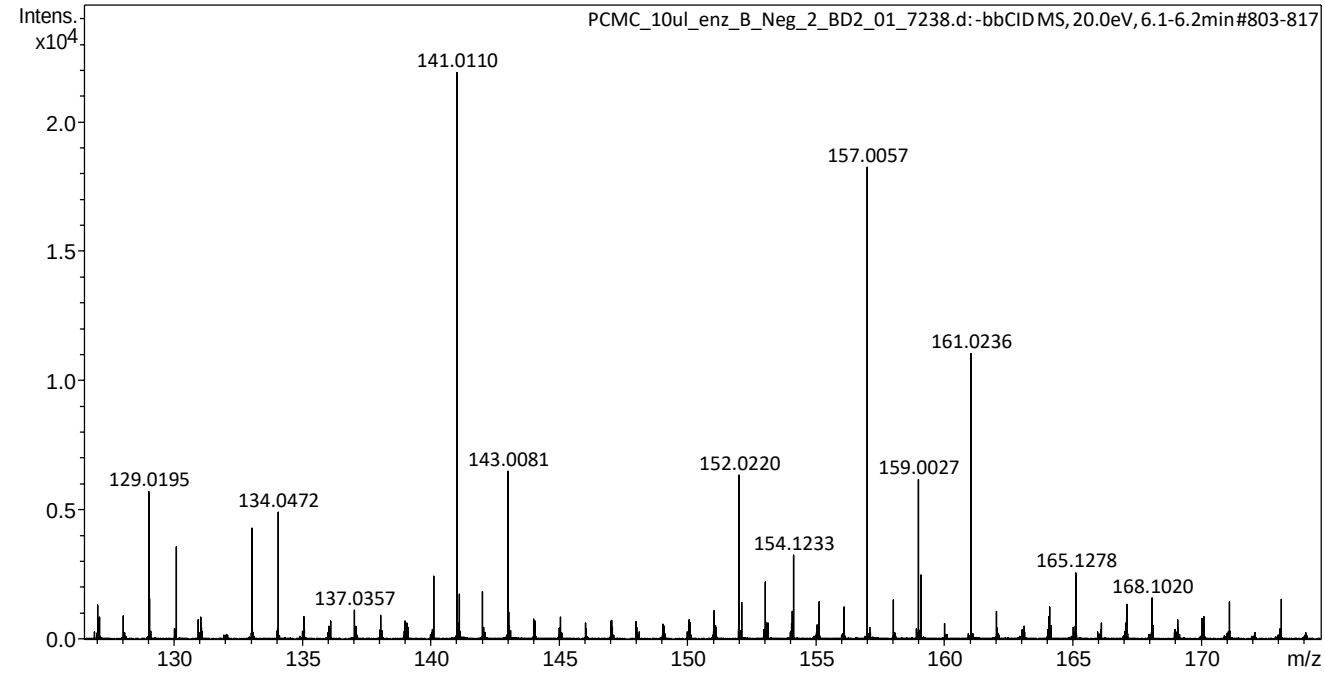

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Analysis Info

Acquisition Date 11/11/2016 01:26:22

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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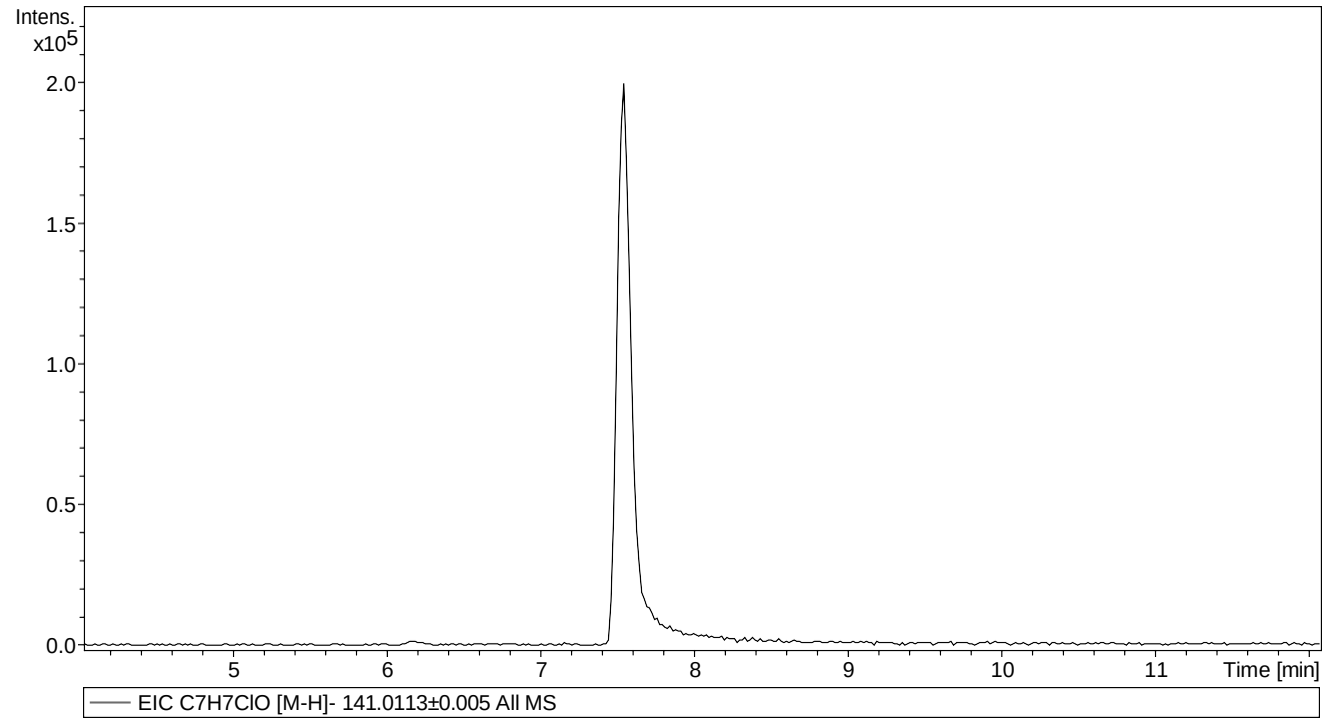

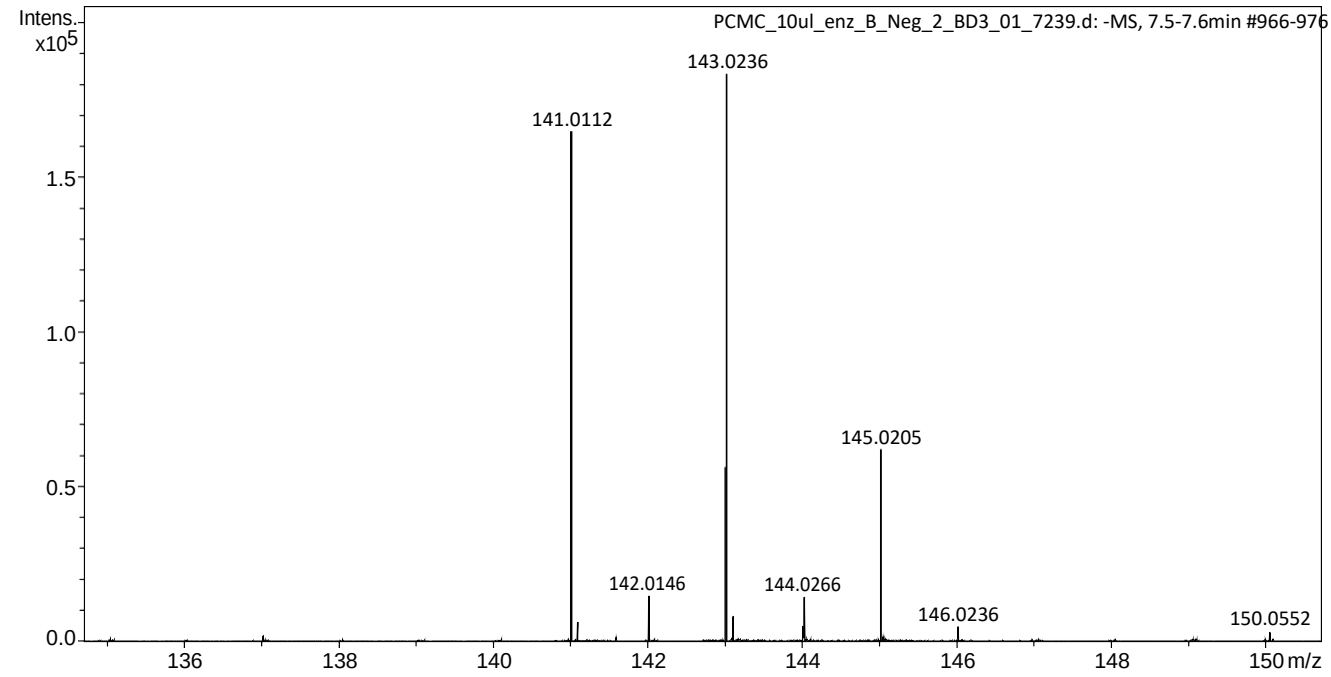

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Analysis Info

Acquisition Date 11/11/2016 01:26:22

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

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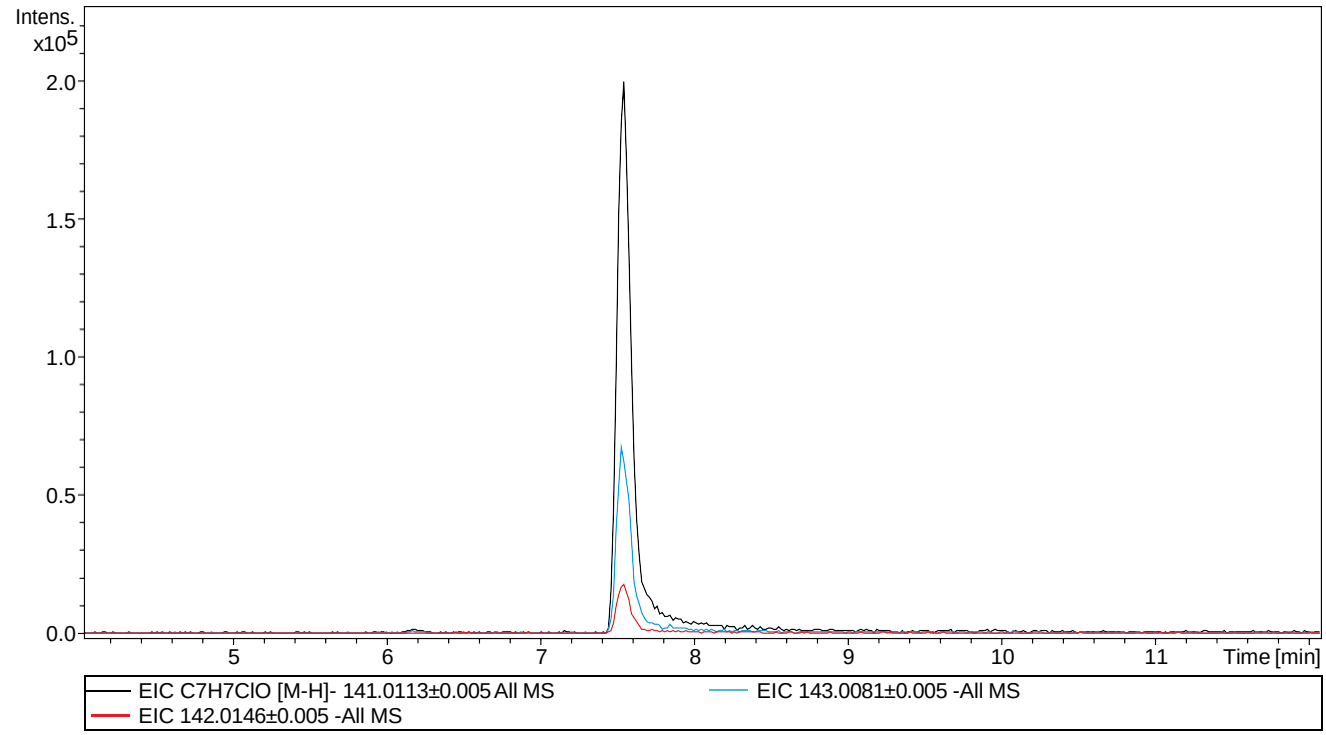
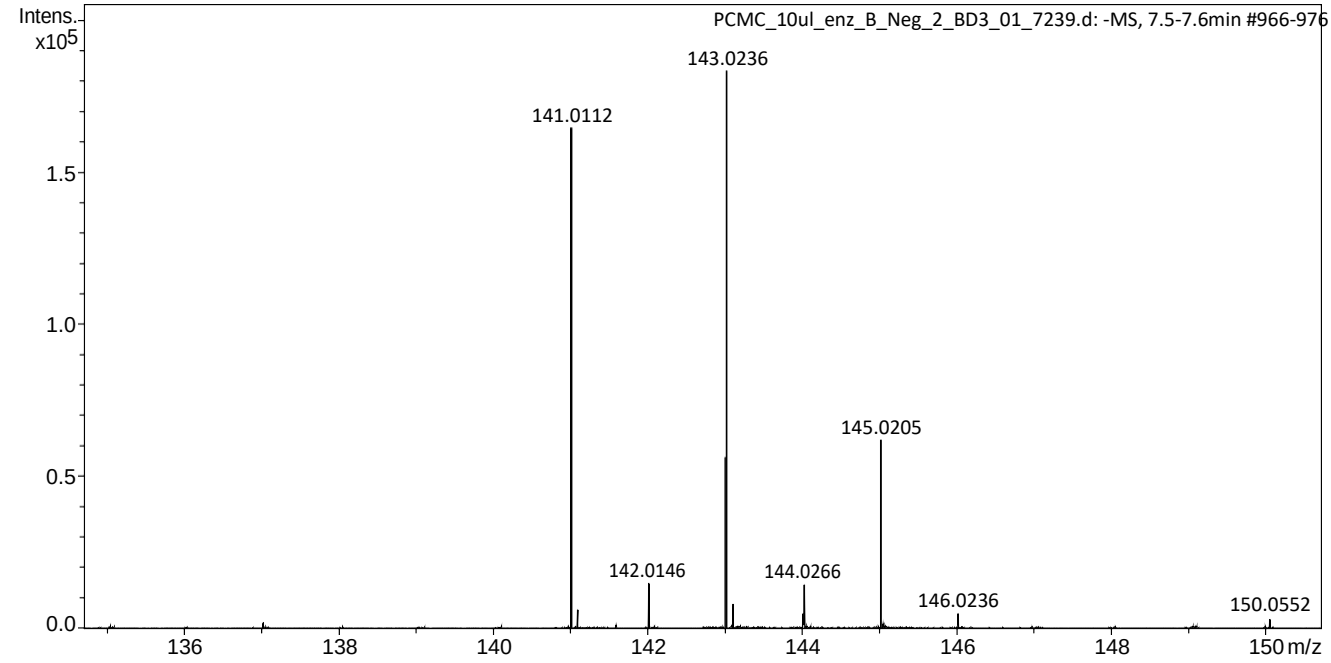
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Analysis Info

Acquisition Date 11/11/2016 01:05:06

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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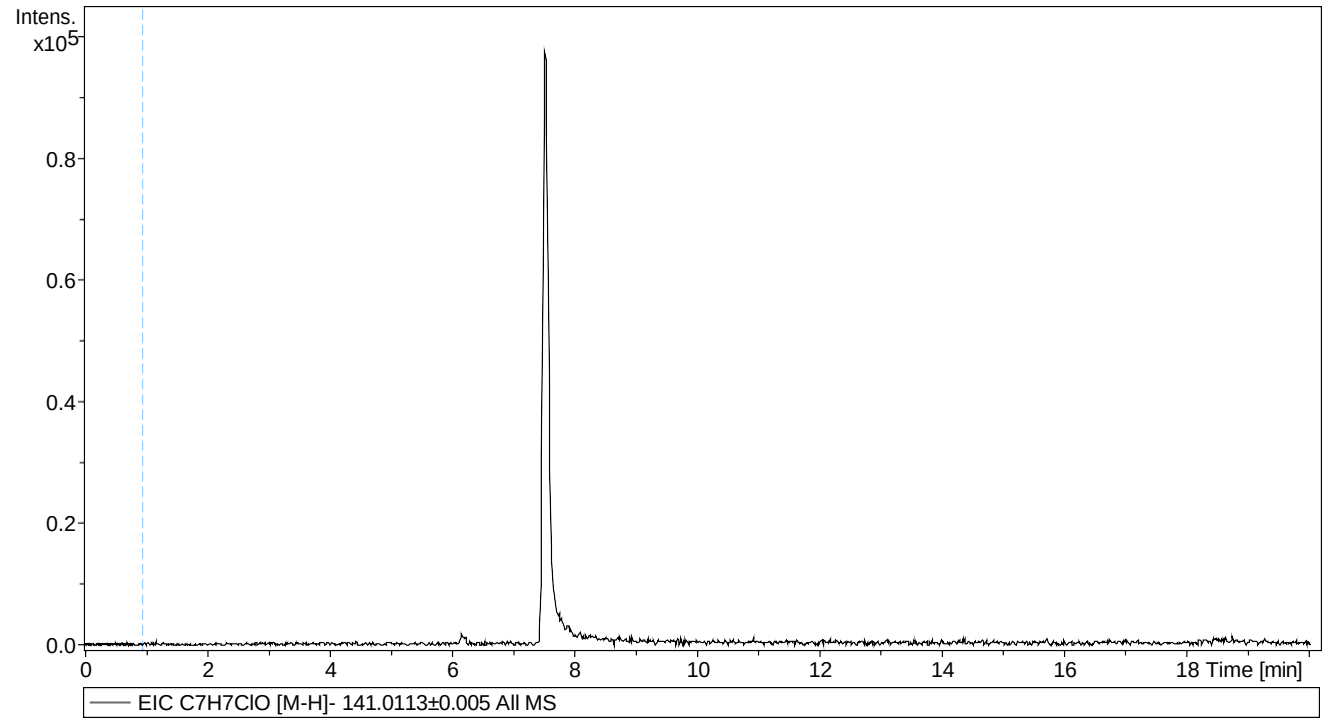

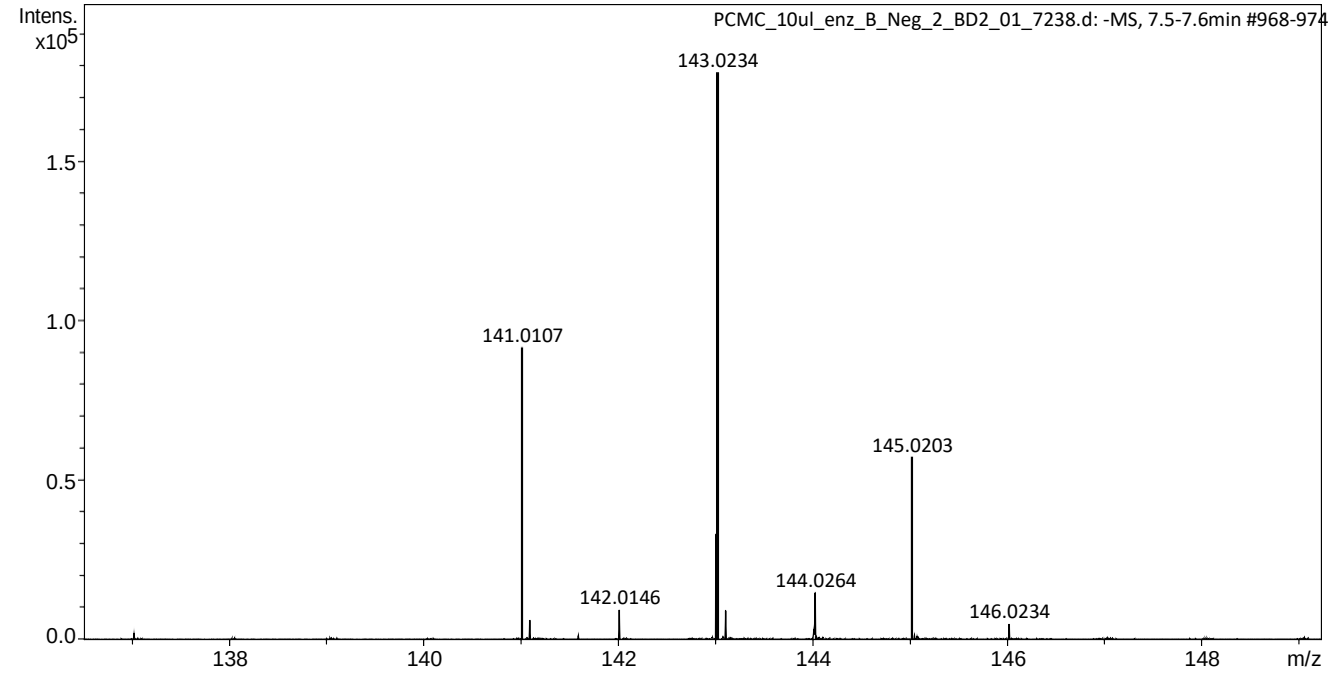

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Analysis Info

Acquisition Date 11/11/2016 01:05:06

Operator BDAL@DE

Sample Name PCMC_10ul_enz_B_Neg_2

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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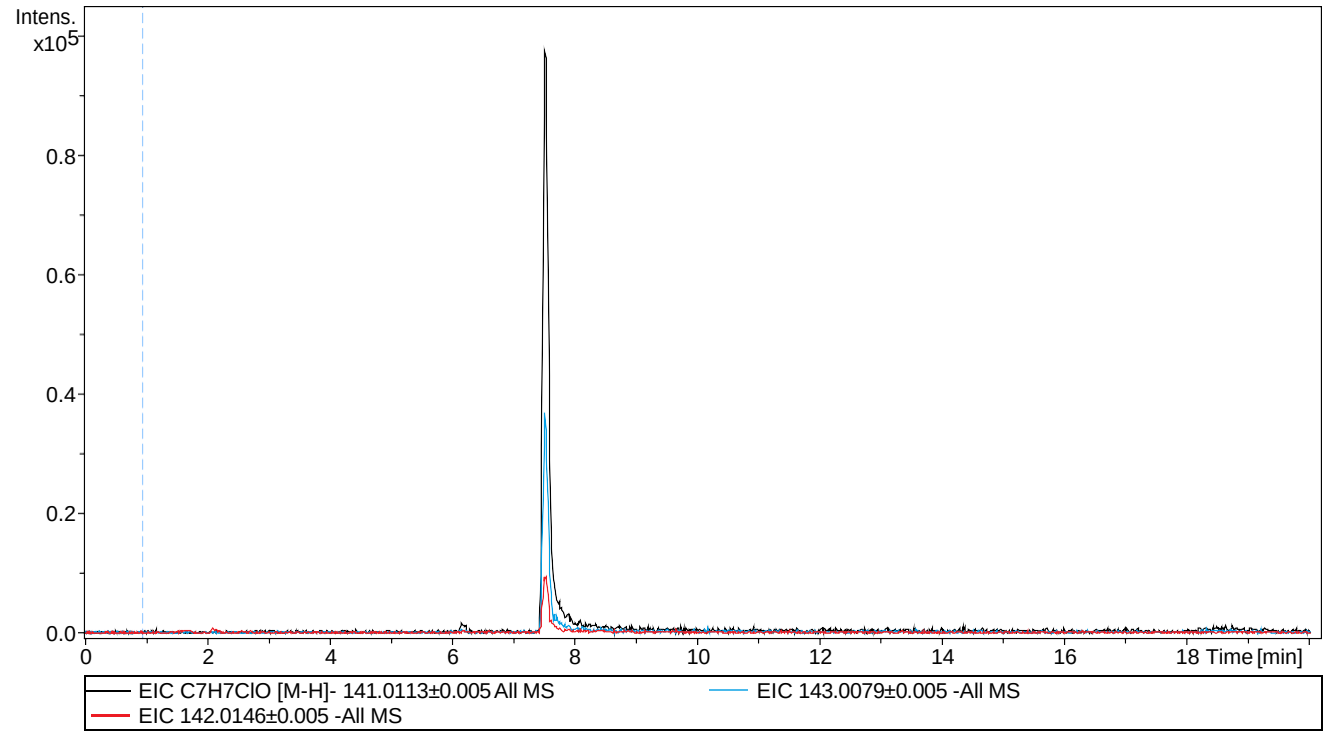

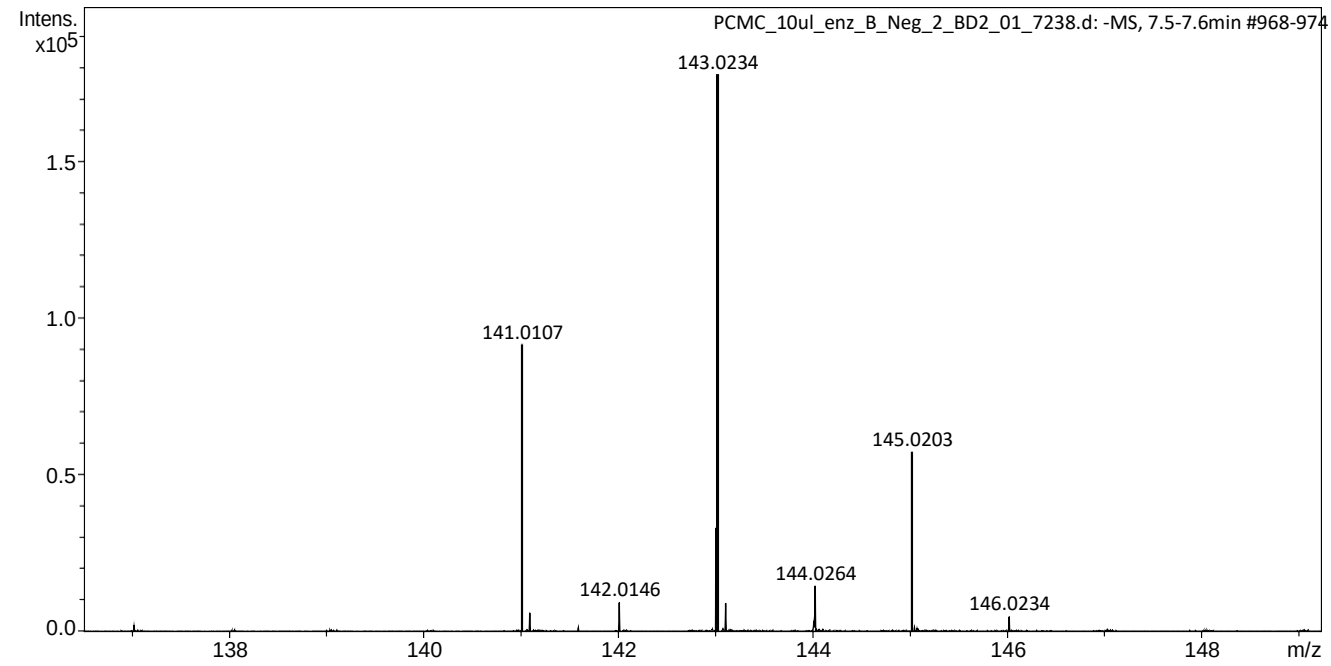

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Analysis Info

Acquisition Date 6/18/2015 1:09:28 PM

Sample Name 4-Cl-3-Me_Blank_neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

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Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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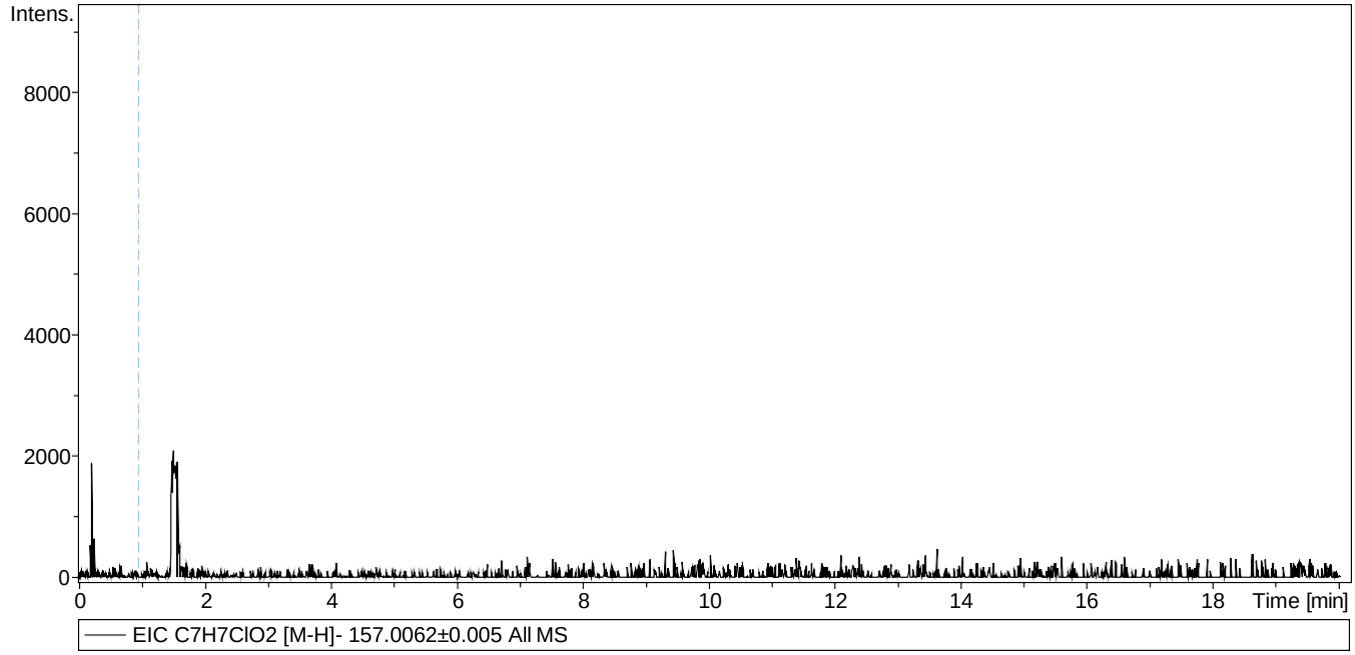
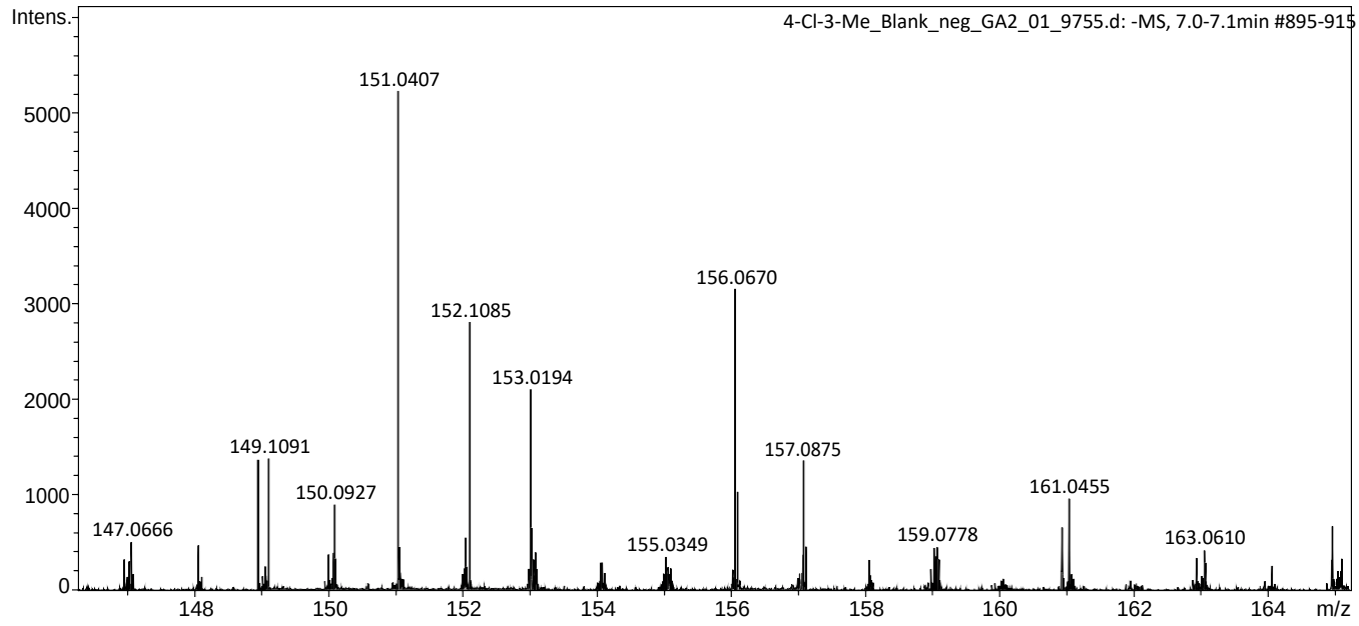




Display Report

Analysis Info

Acquisition Date 10/11/2016 19:46:30

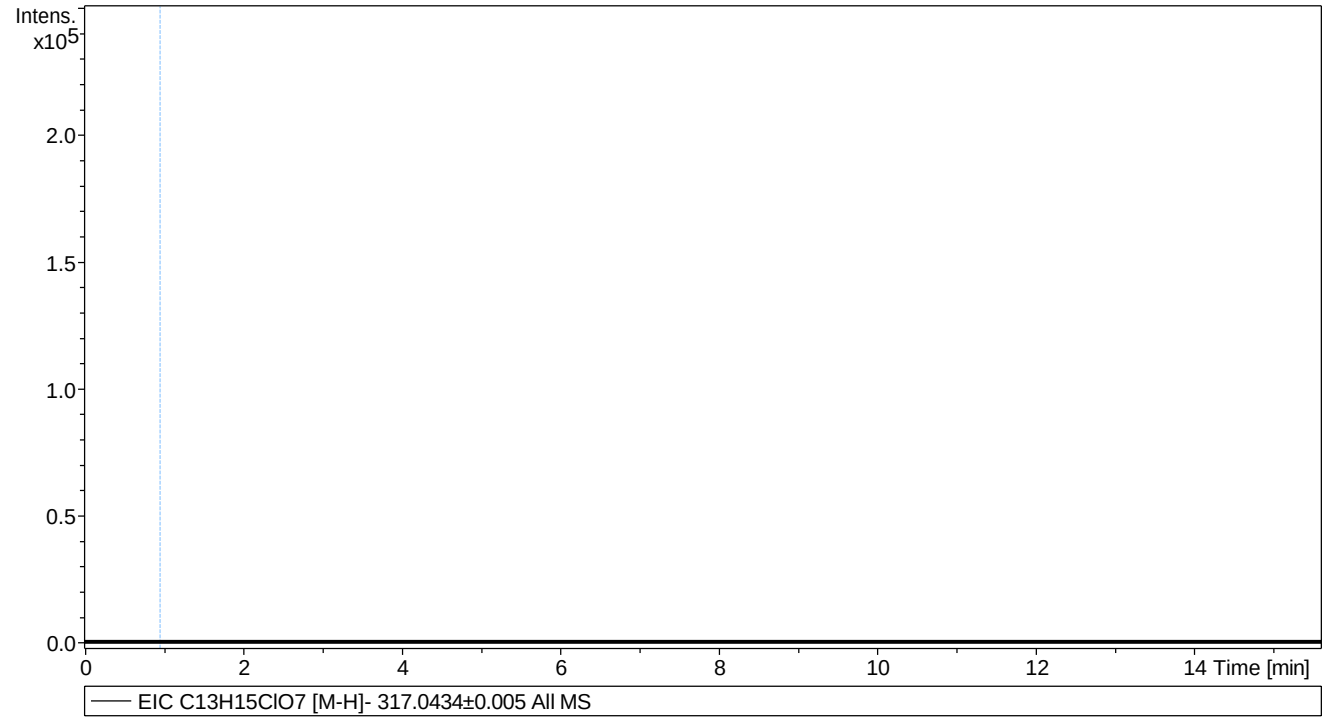
Operator BDAL@DE

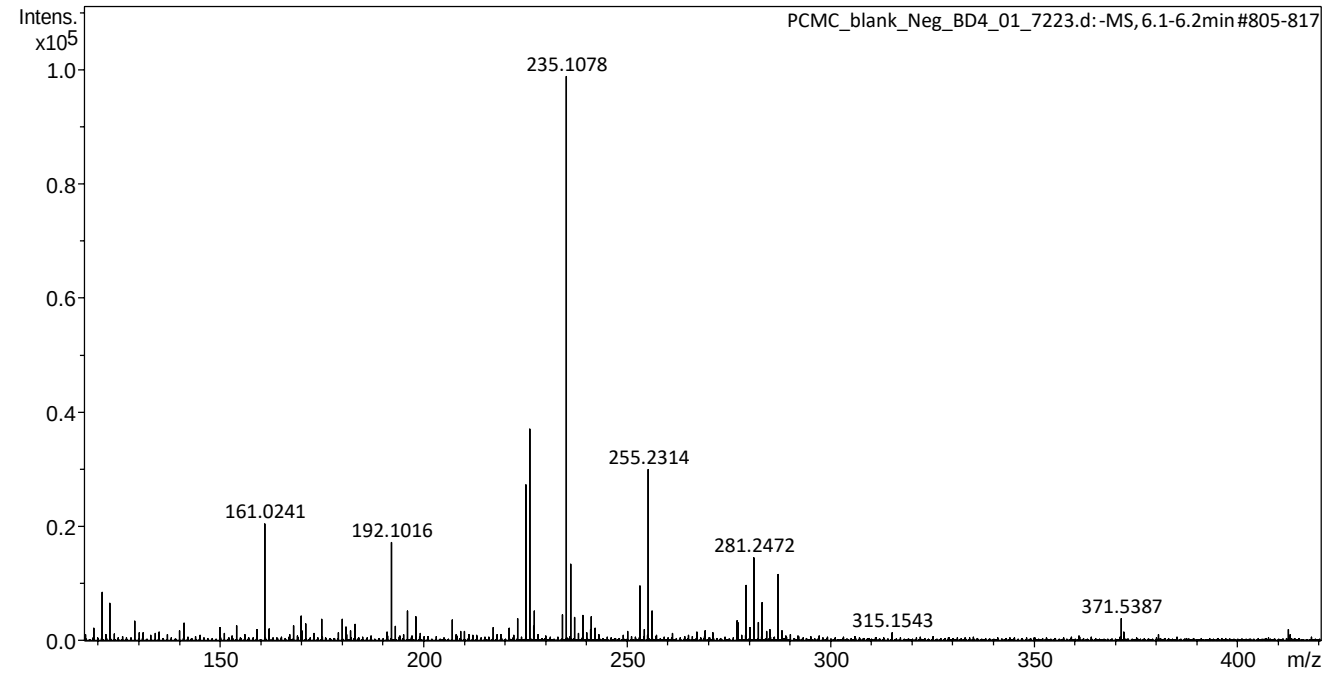
Sample Name PCMC_blank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 6/18/2015 1:09:28 PM

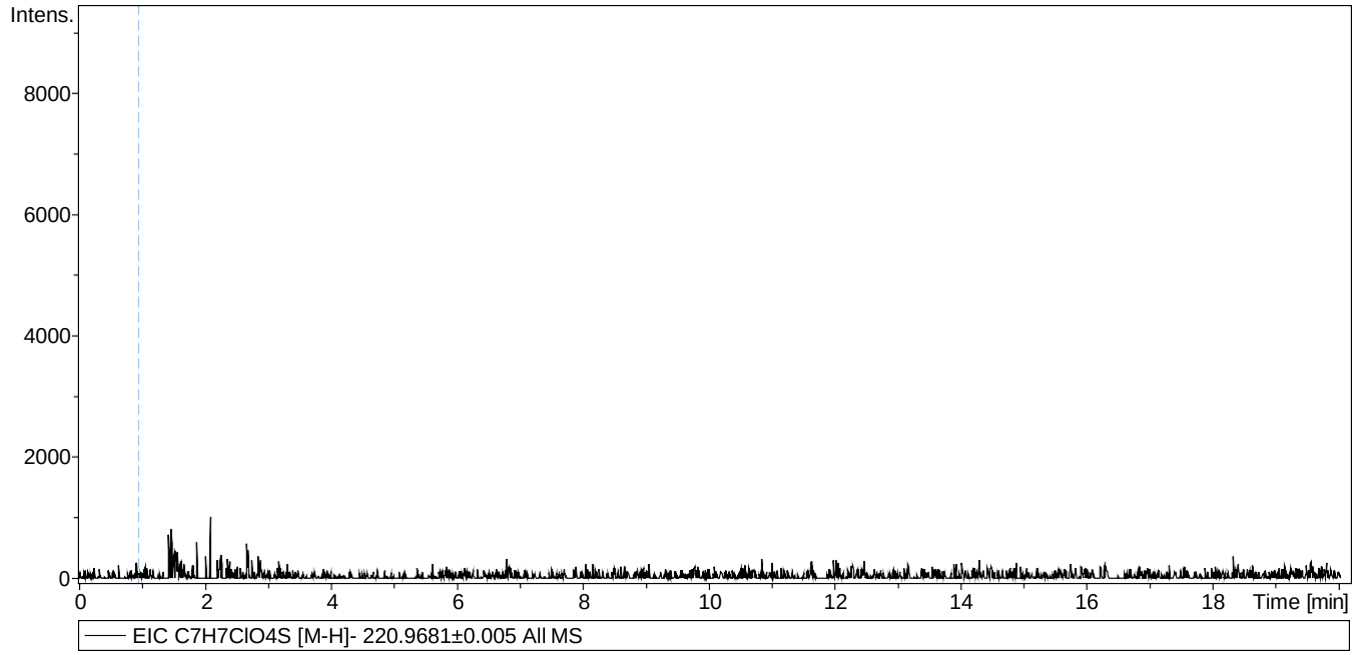
Sample Name 4-Cl-3-Me_Blank_neg

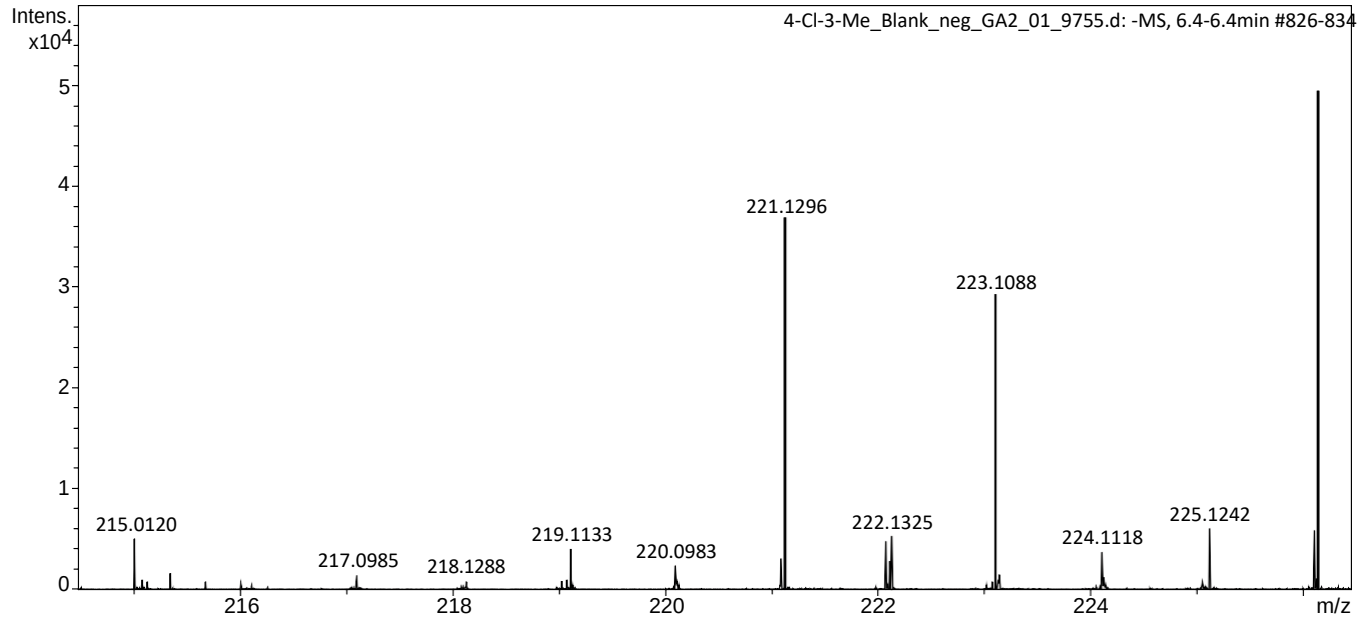
Operator CCAF

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





4-Cl-3-Me_Blank_neg_GA2_01_9755.d

Display Report

Analysis Info

Acquisition Date 6/18/2015 1:09:28 PM

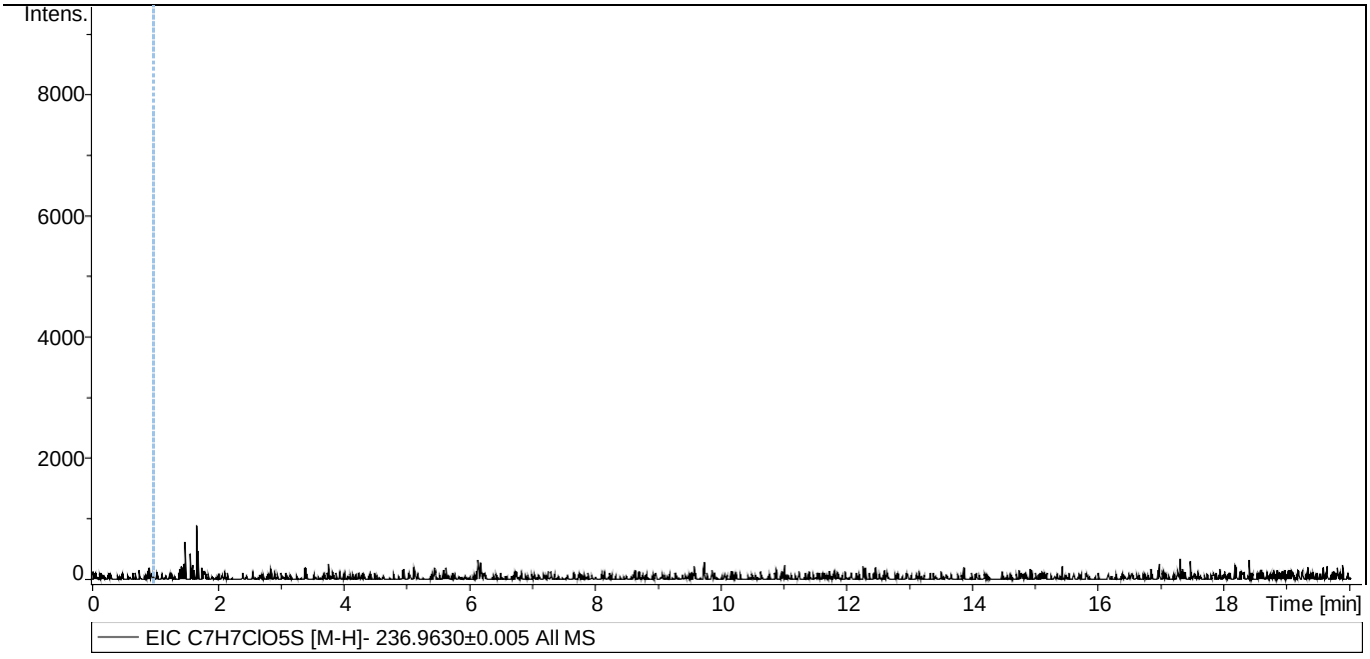
Sample Name 4-Cl-3-Me_Blank_neg

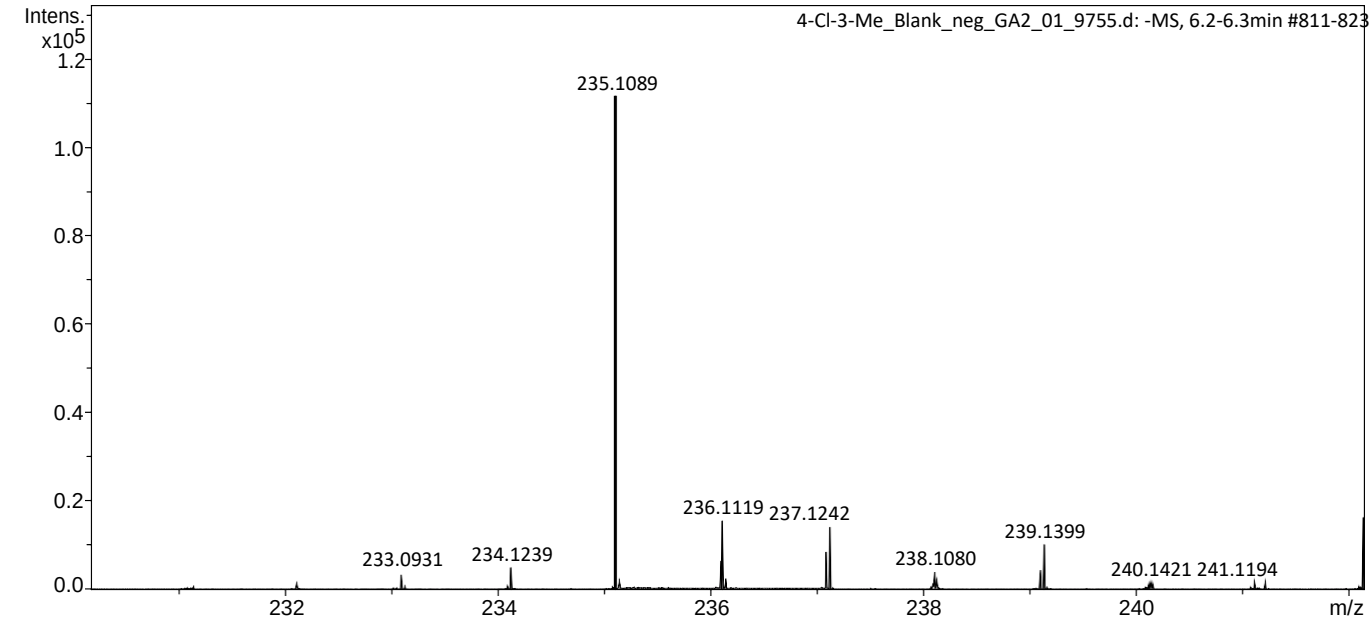
Operator CCAF

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





4-Cl-3-Me_Blank_neg_GA2_01_9755.d

Display Report

Analysis Info

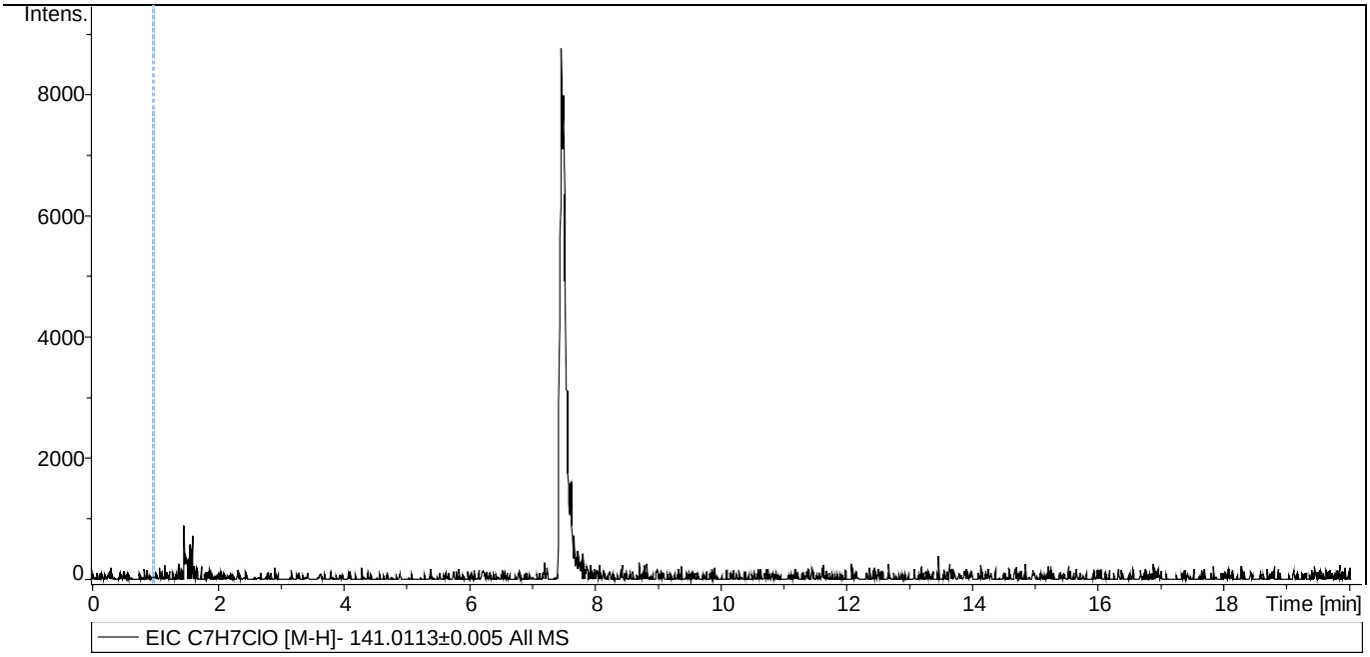
Acquisition Date 6/18/2015 1:09:28 PM

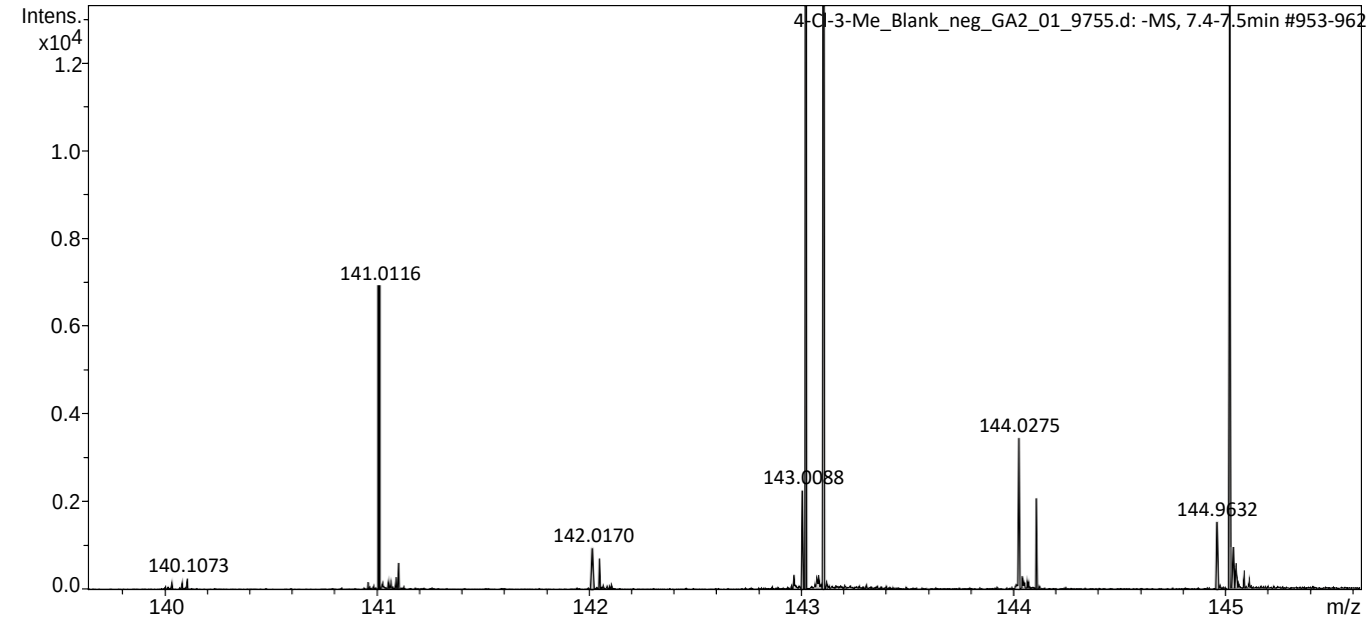
Sample Name 4-Cl-3-Me_Blank_neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





4-Cl-3-Me_Blank_neg_GA2_01_9755.d

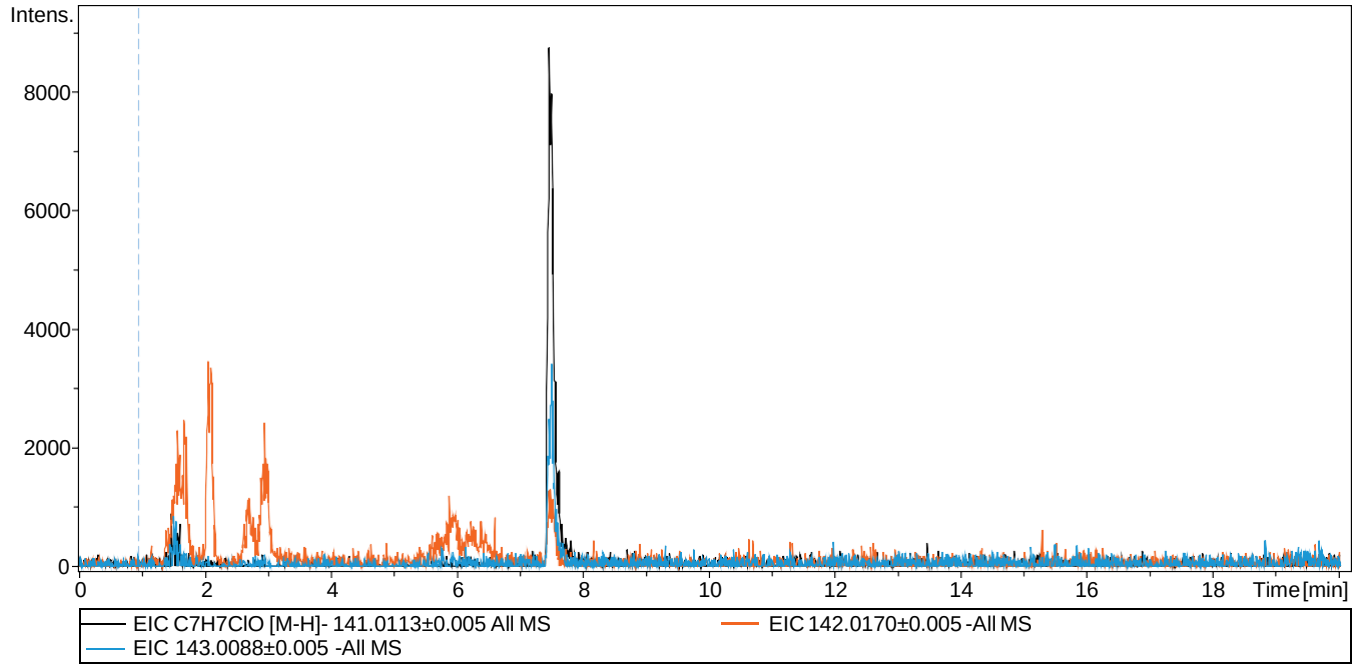
Display Report

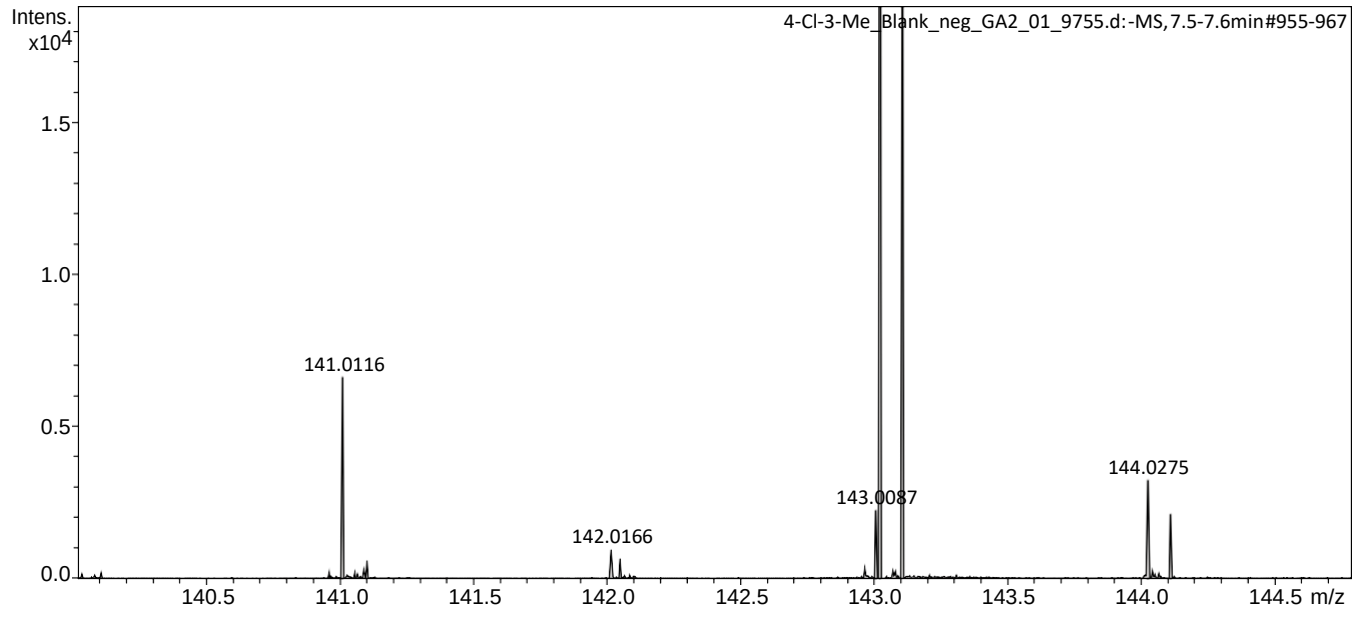
Analysis Info

Acquisition Date	6/18/2015 1:09:28 PM		
Sample Name	4-Cl-3-Me_Blank_neg	Operator	CCAF
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





4-Cl-3-Me_Blank_neg_GA2_01_9755.d

Display Report

Analysis Info

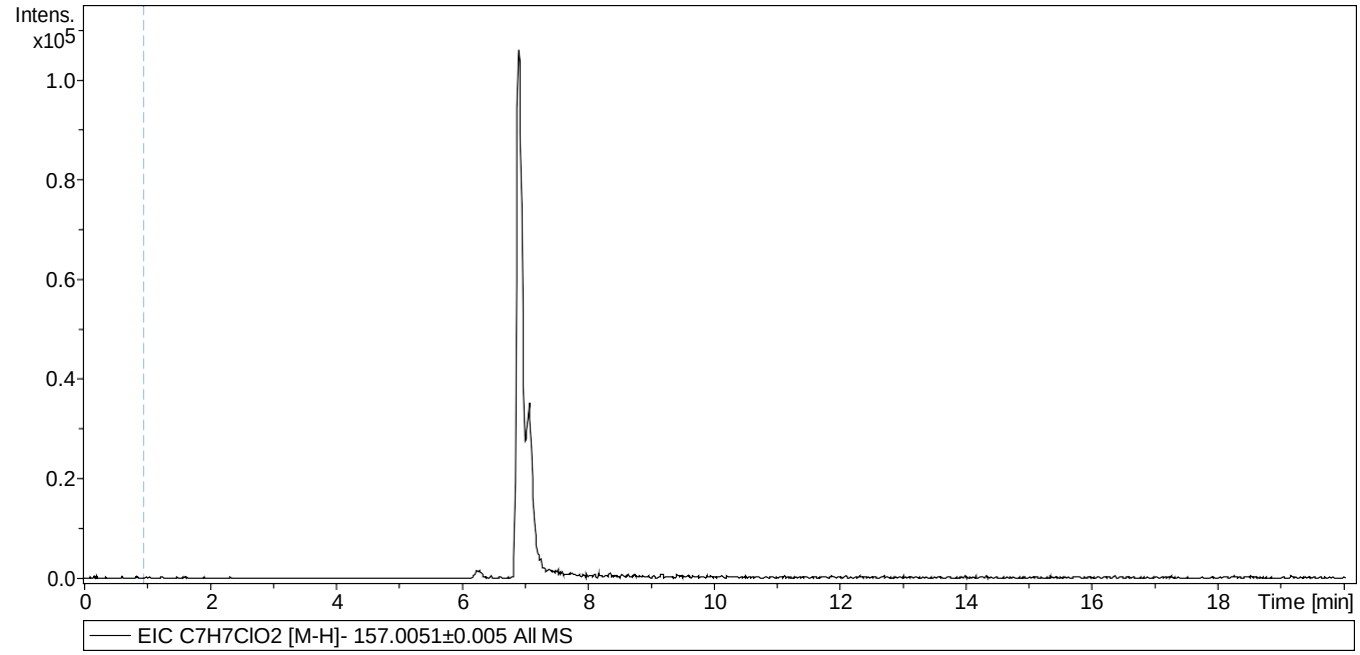
Acquisition Date 1/12/2016 8:13:51 PM

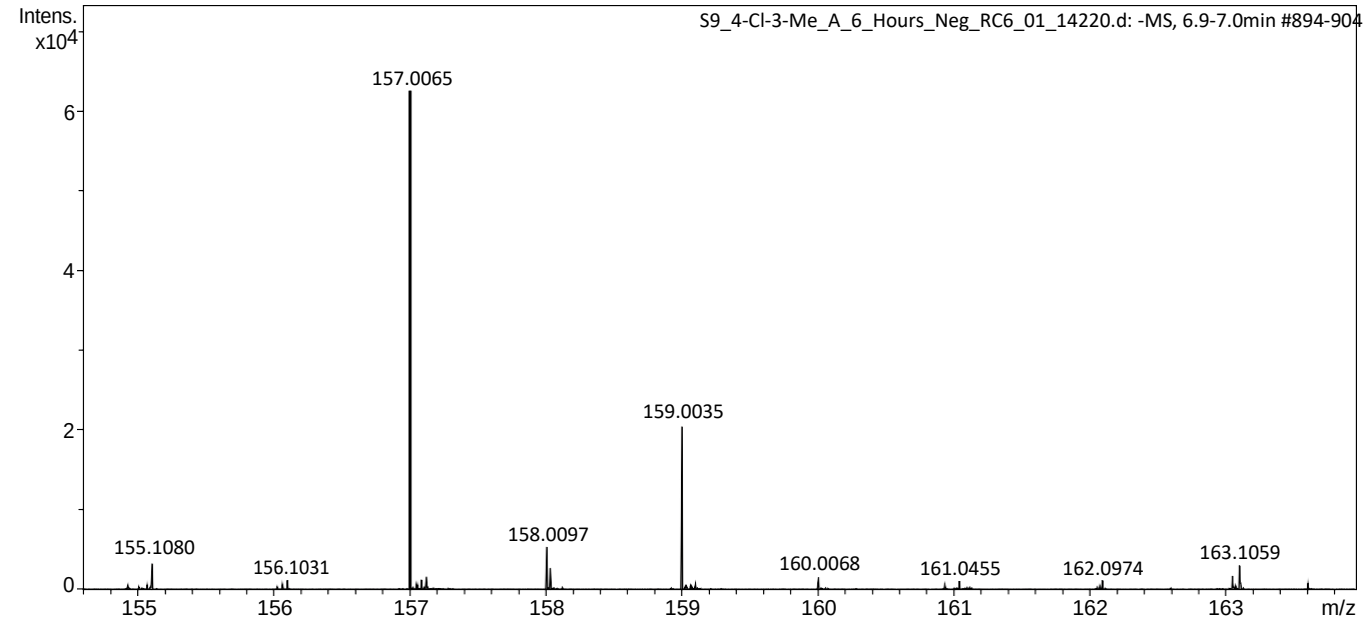
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

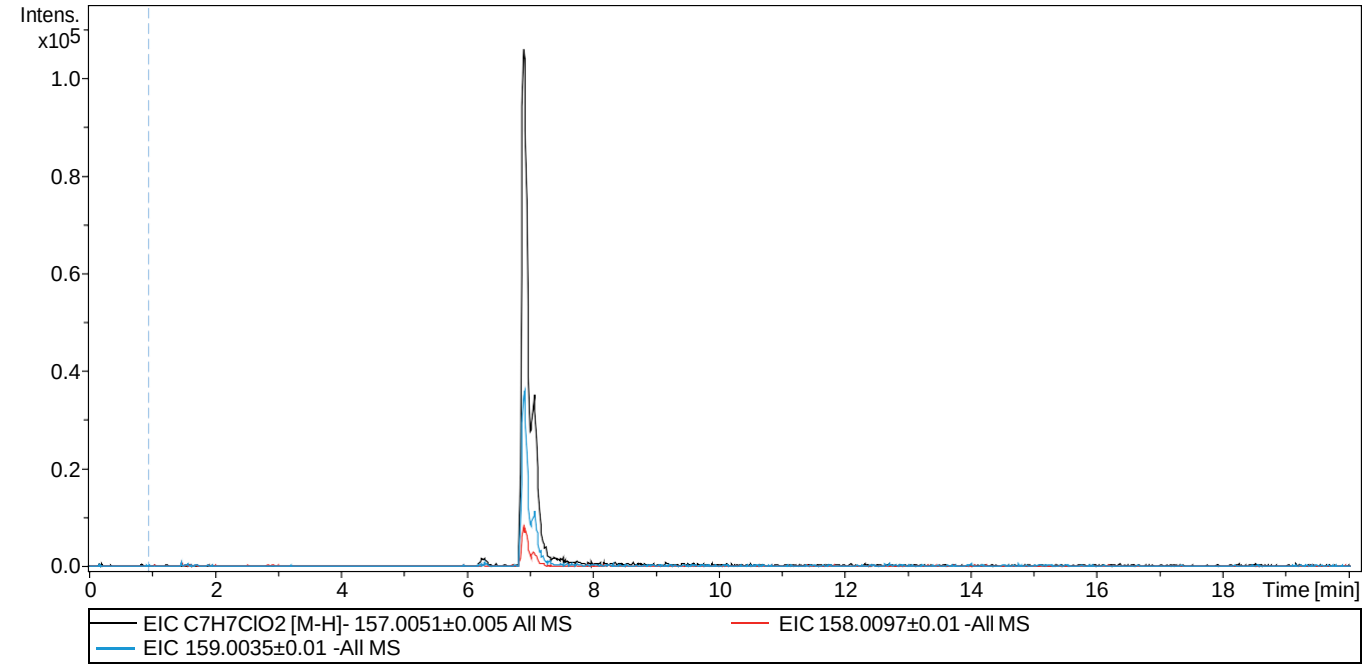
Acquisition Date 1/12/2016 8:13:51 PM

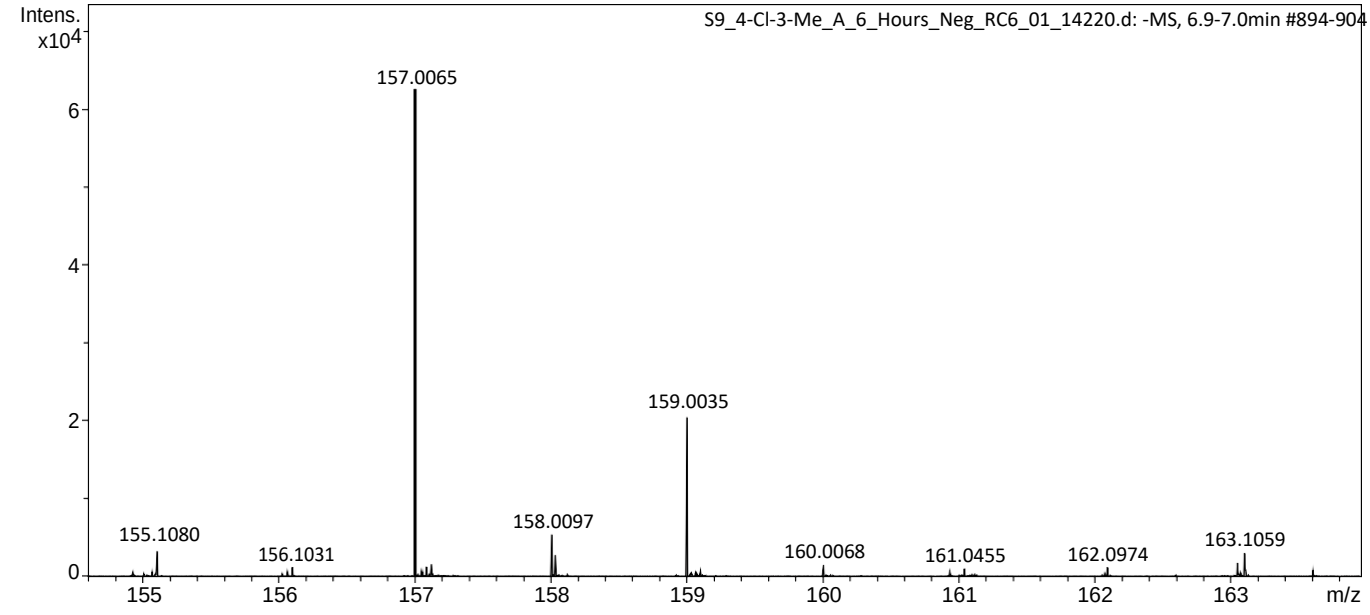
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 20:21:21

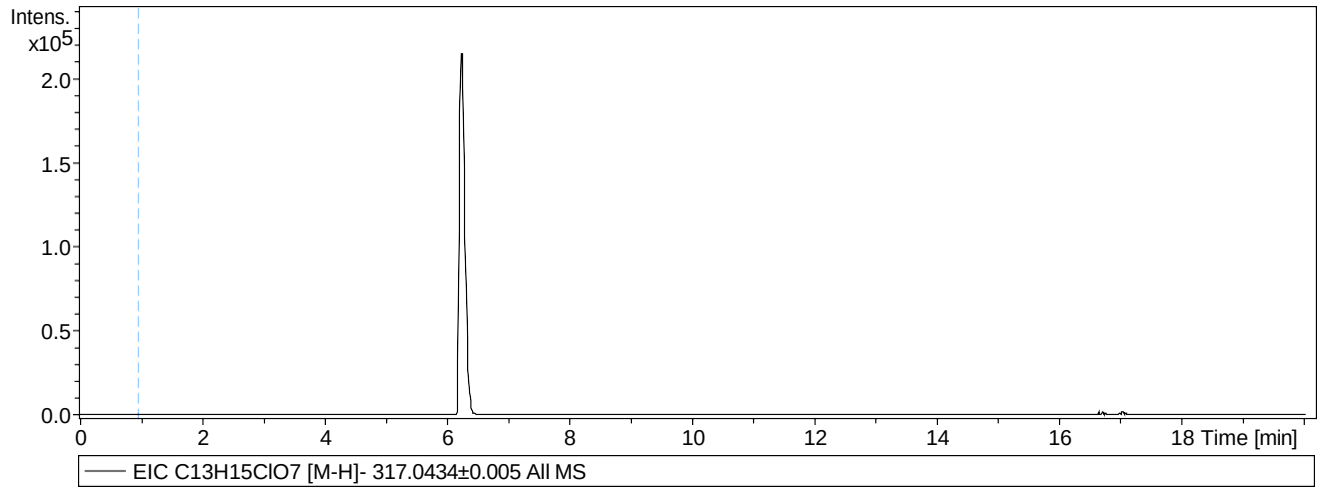
Operator BDAL@DE

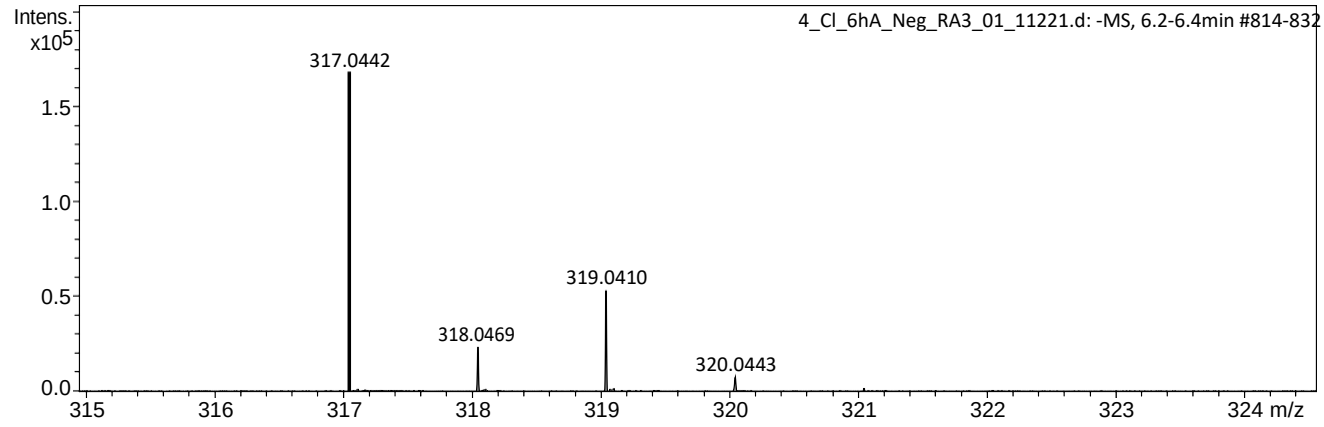
Sample Name 4_Cl_6hA_Neg

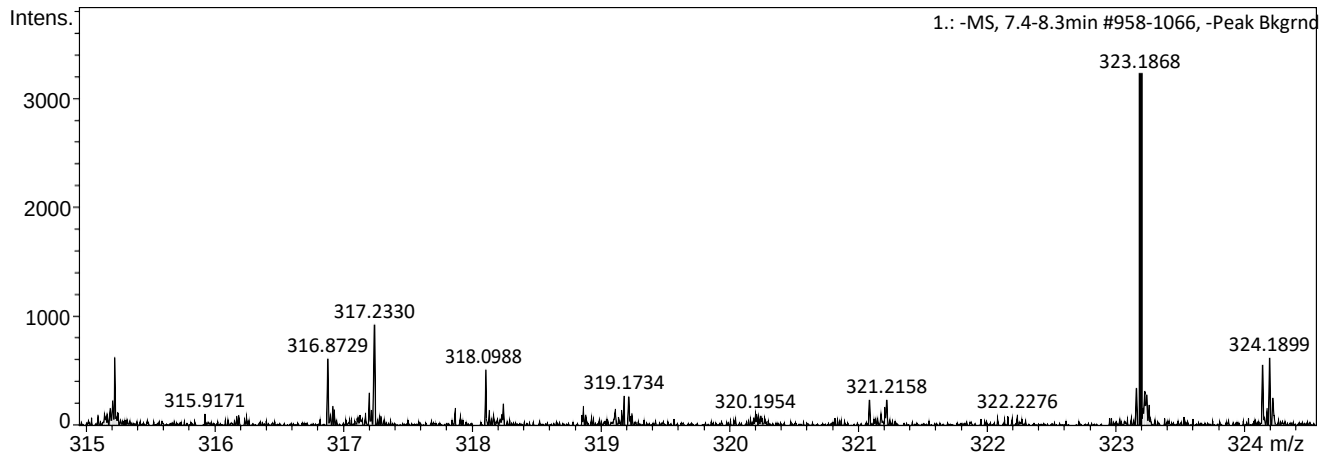
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C







Display Report

Analysis Info

Acquisition Date 15/03/2017 20:21:21

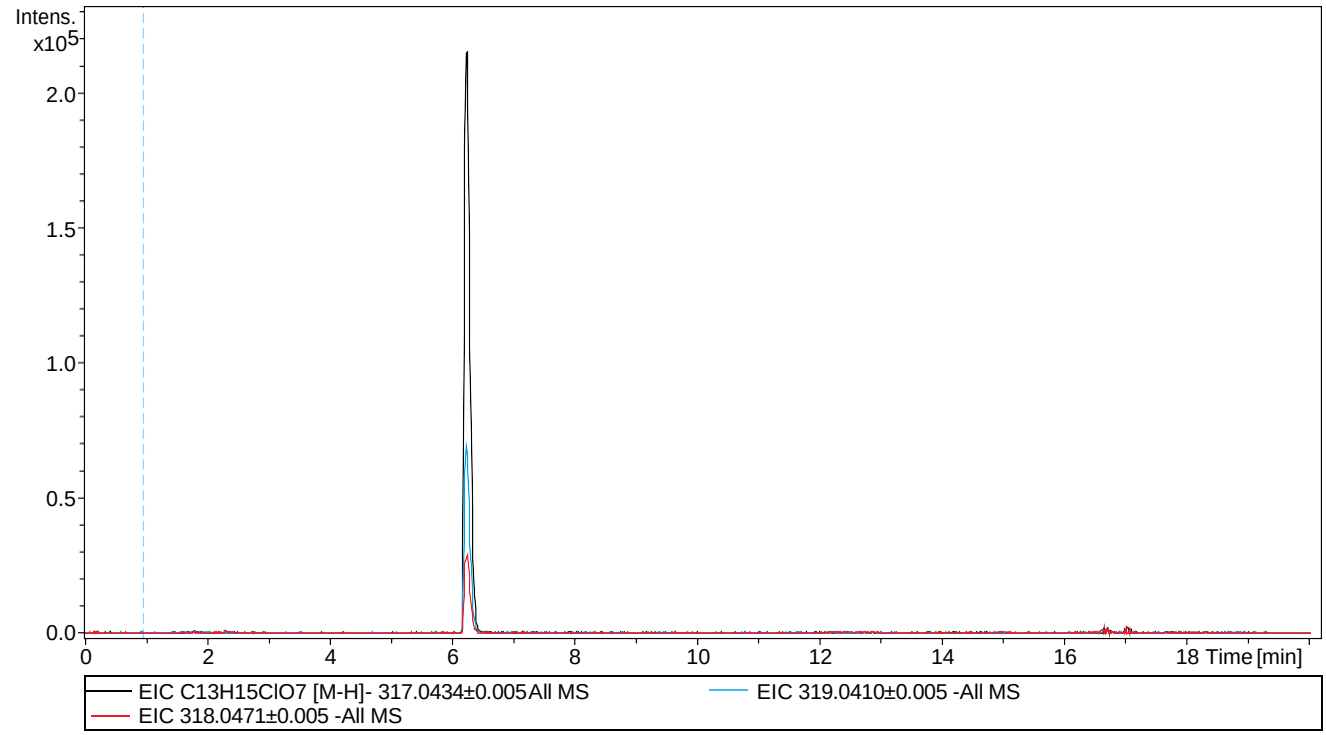
Operator BDAL@DE

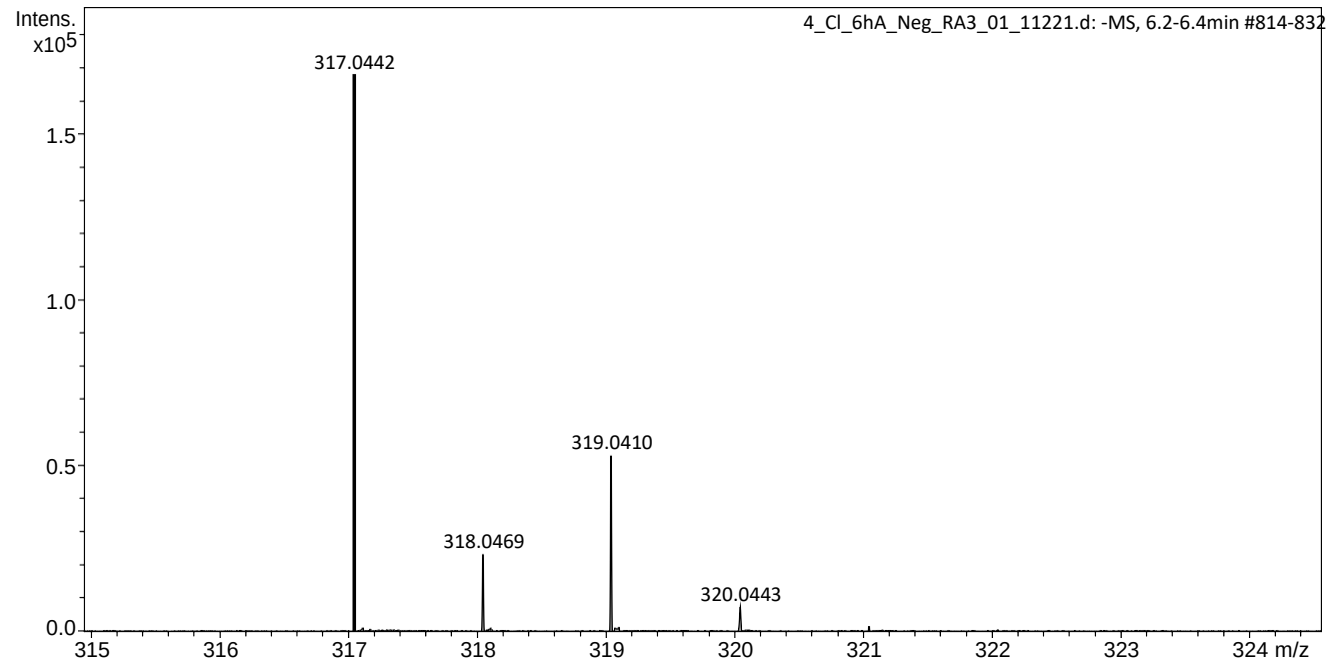
Sample Name 4_Cl_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 20:21:21

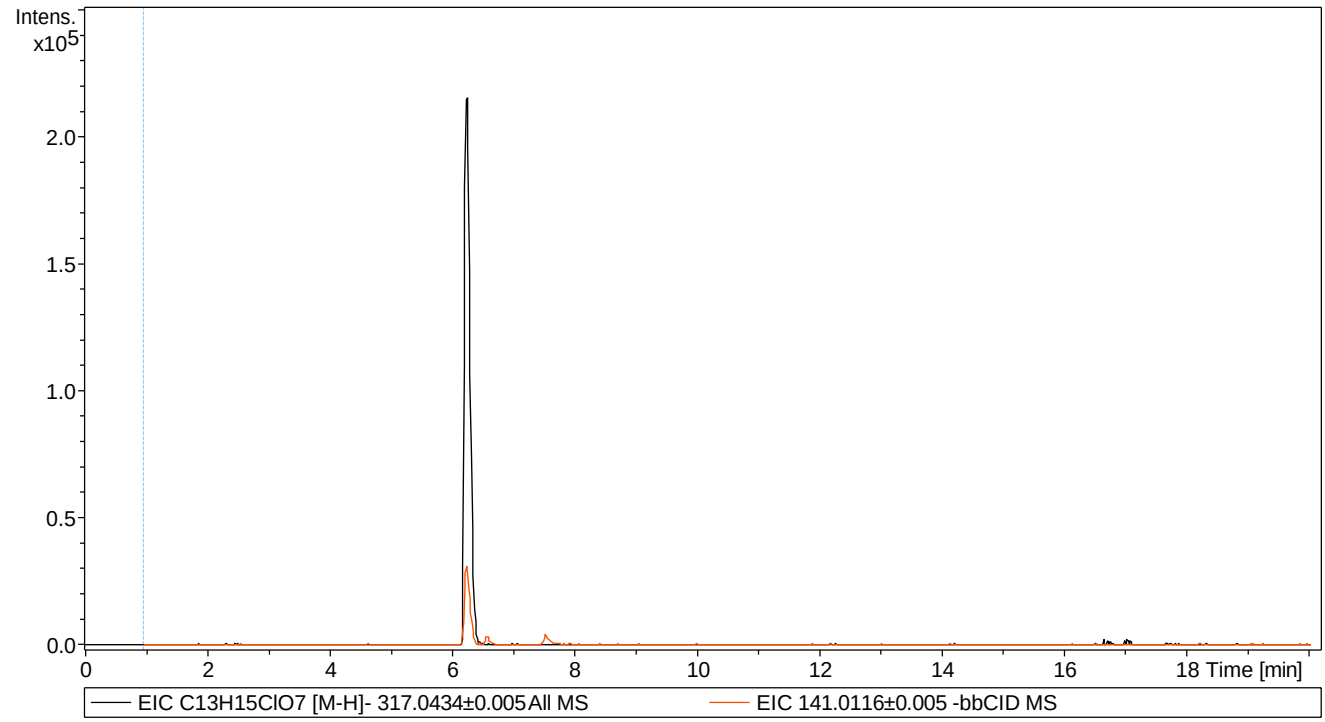
Operator BDAL@DE

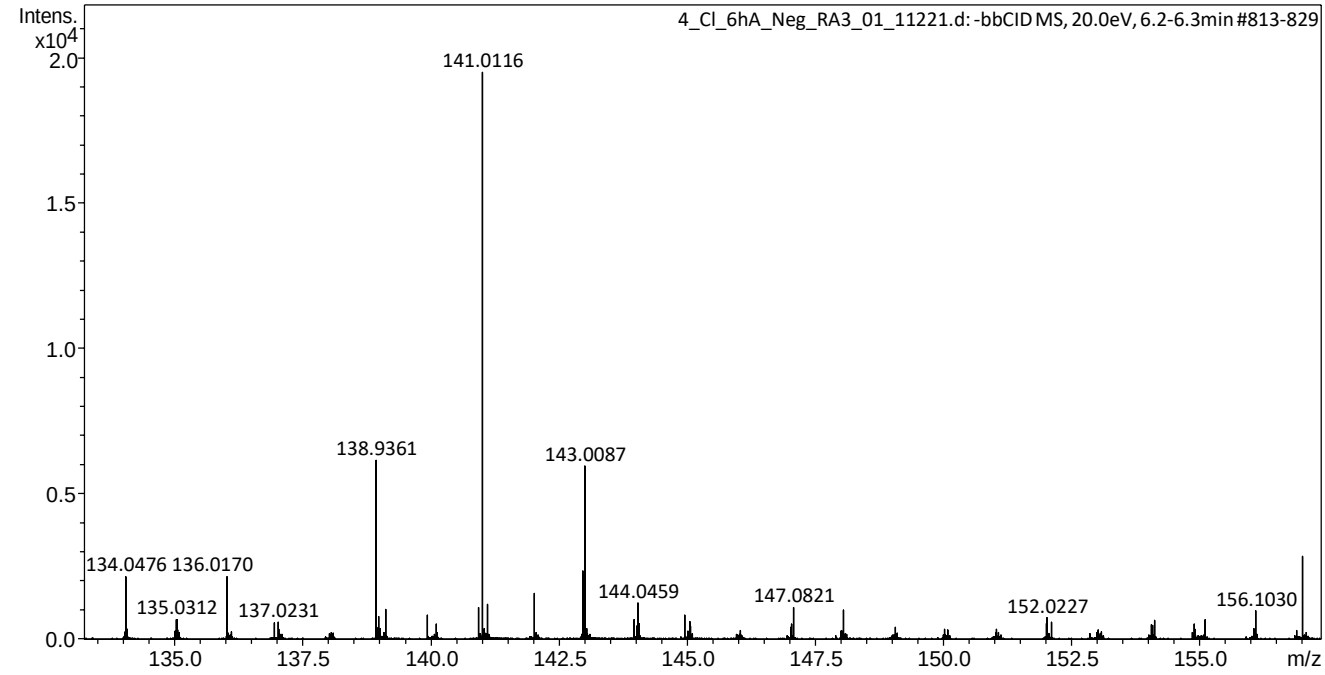
Sample Name 4_Cl_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

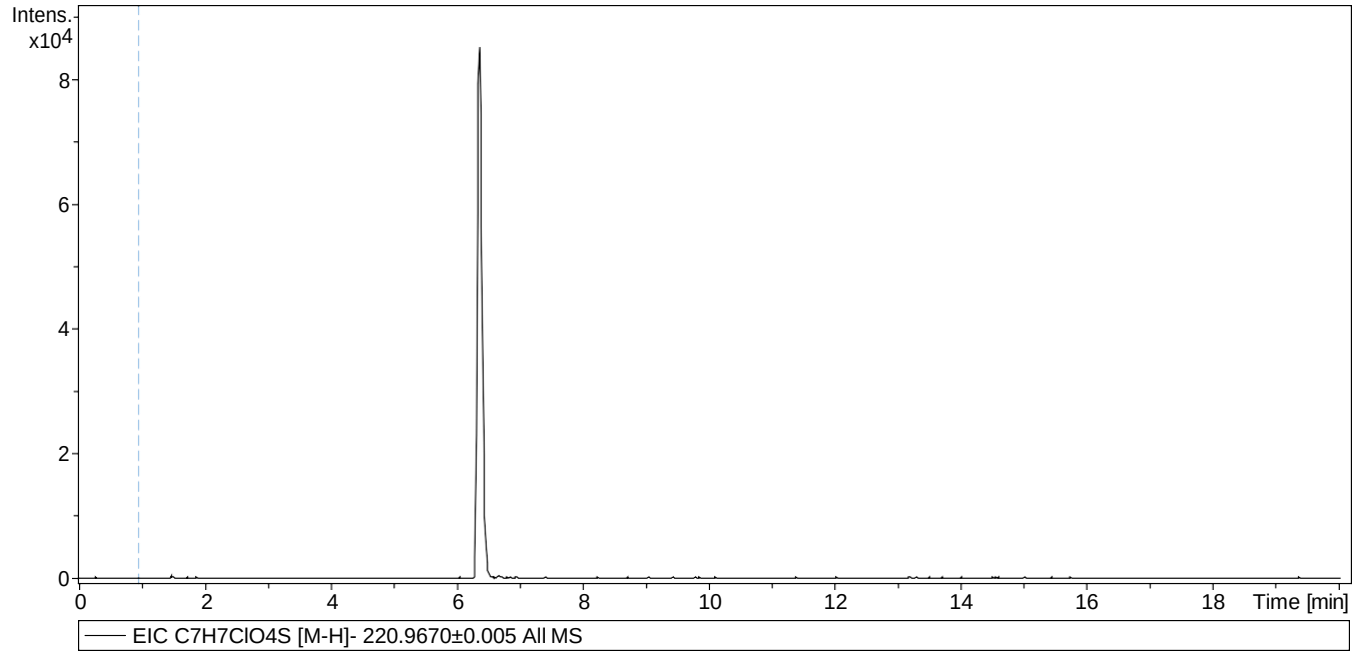
Acquisition Date 1/12/2016 8:13:51 PM

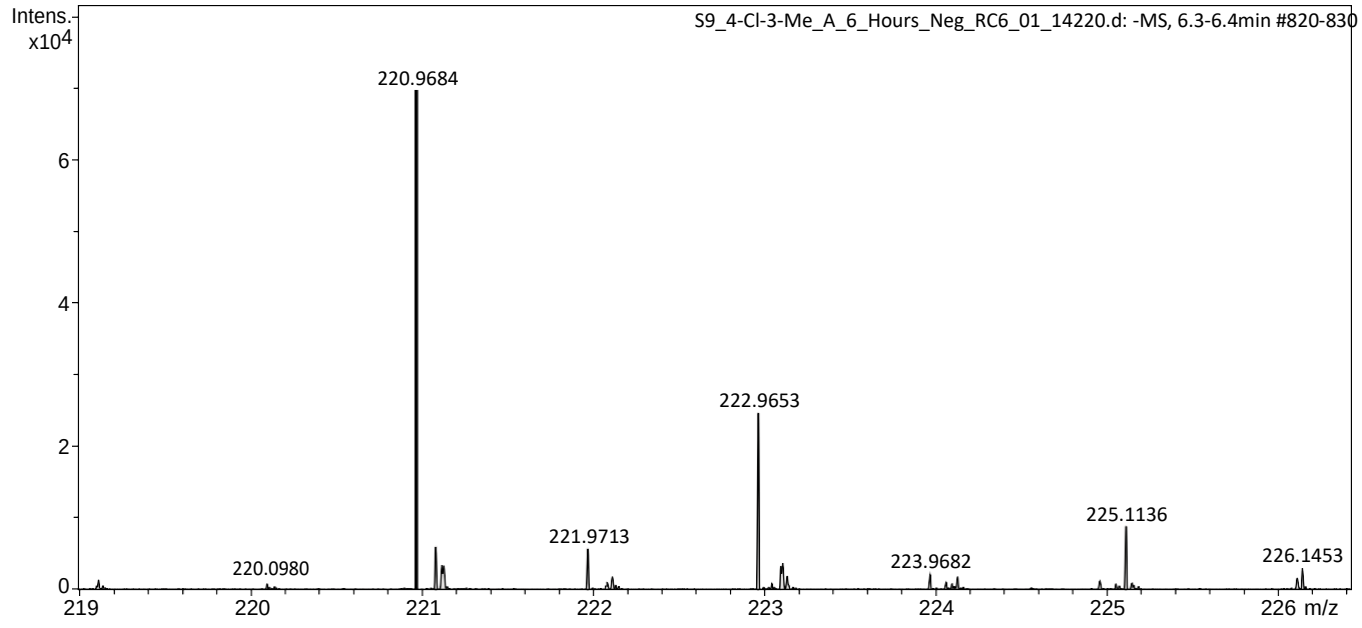
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

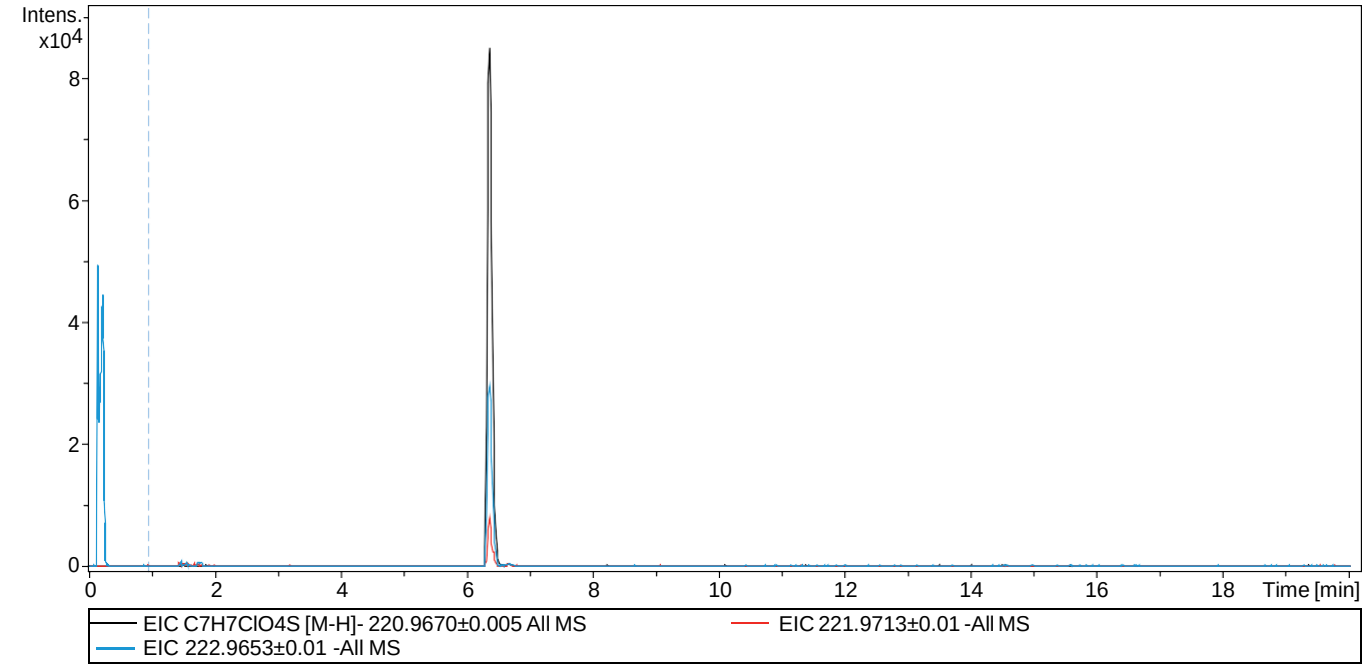
Acquisition Date 1/12/2016 8:13:51 PM

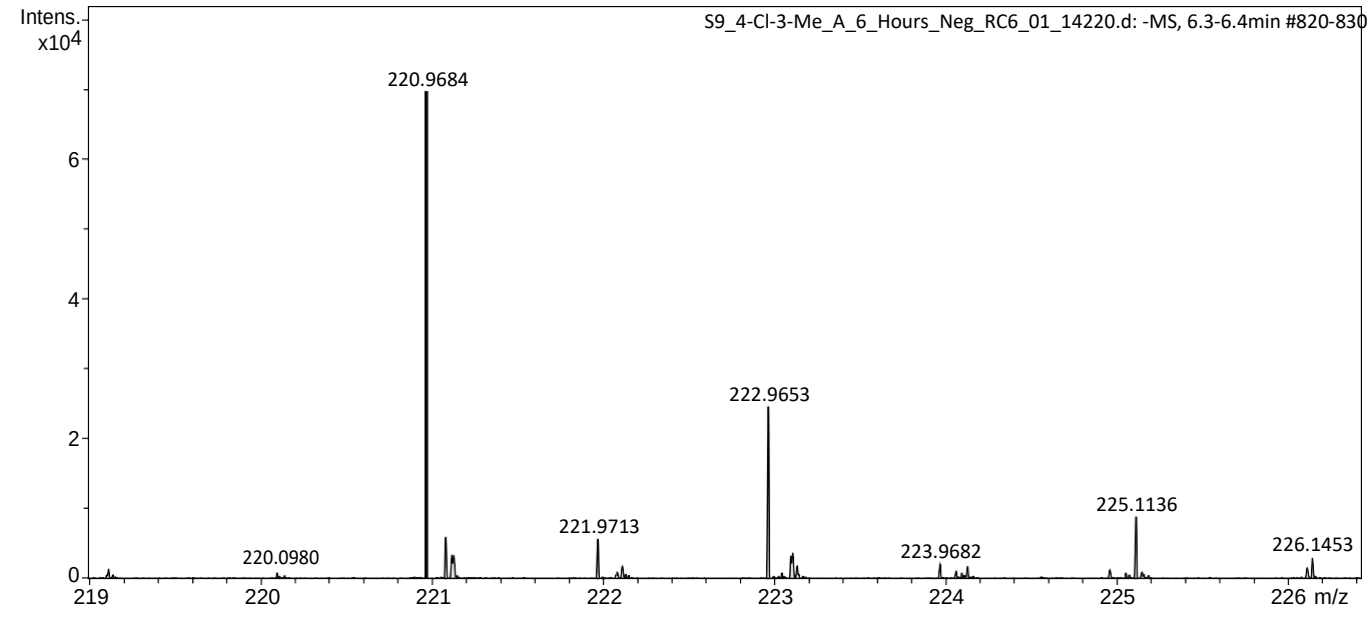
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

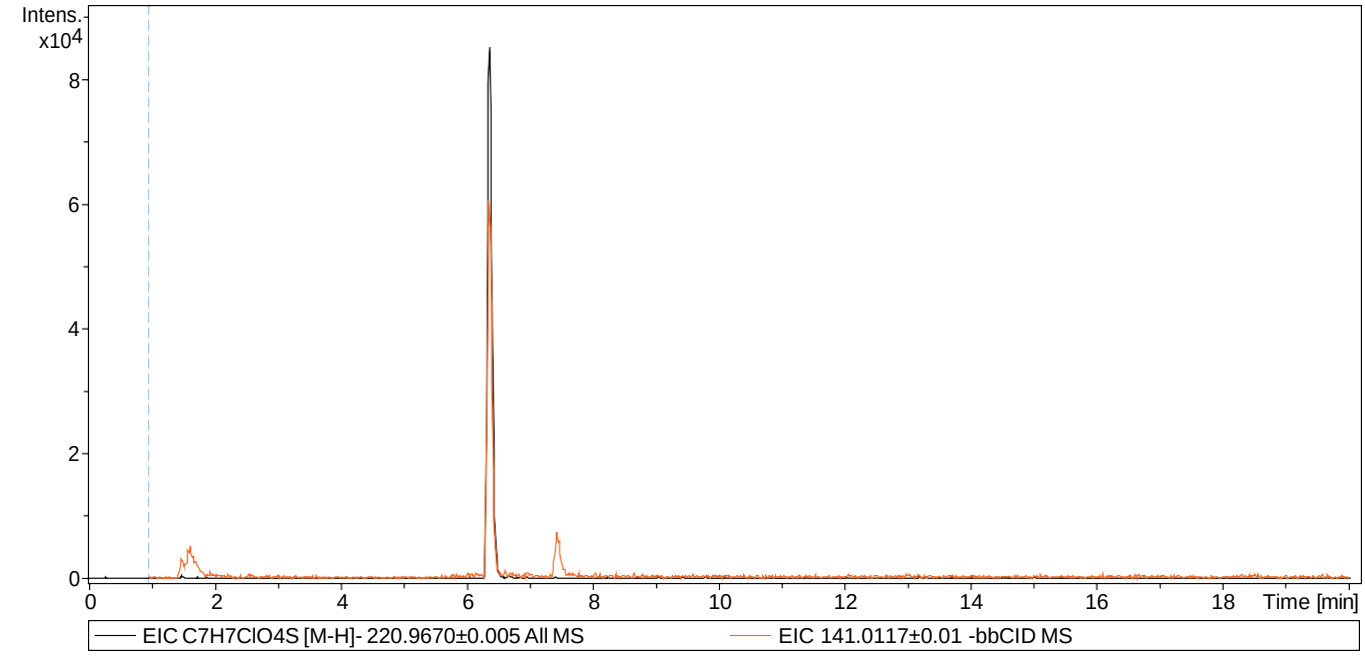
Acquisition Date 1/12/2016 8:13:51 PM

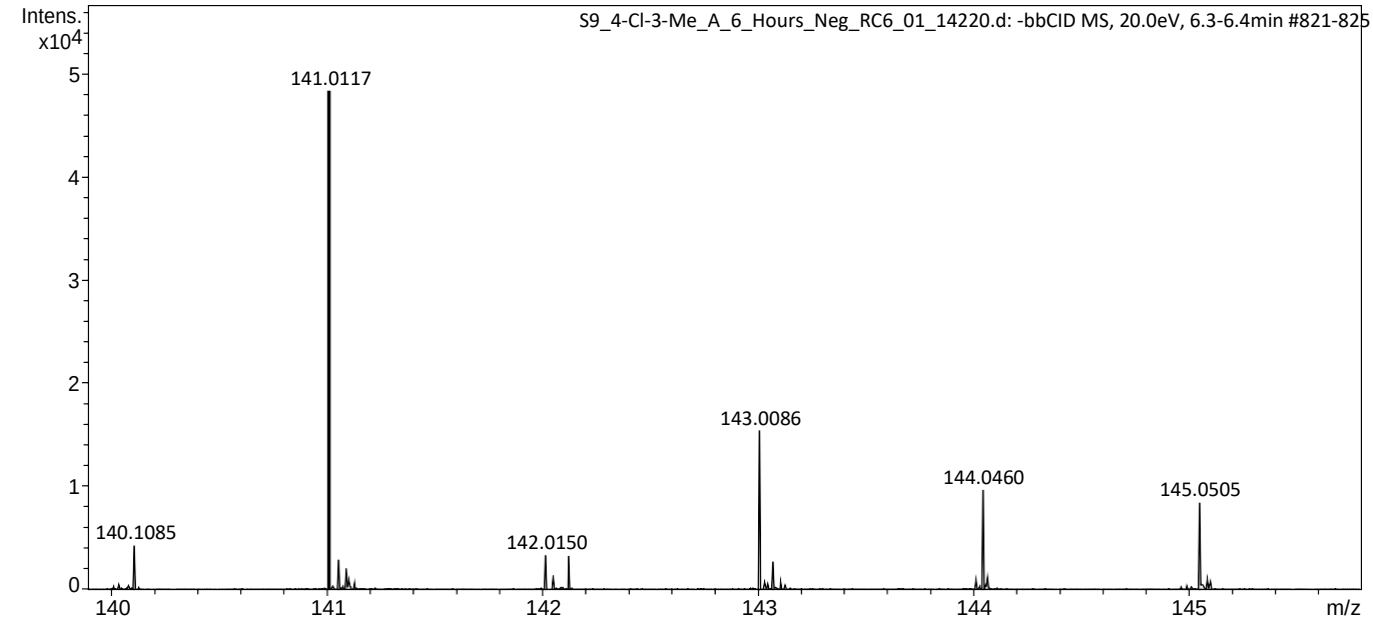
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

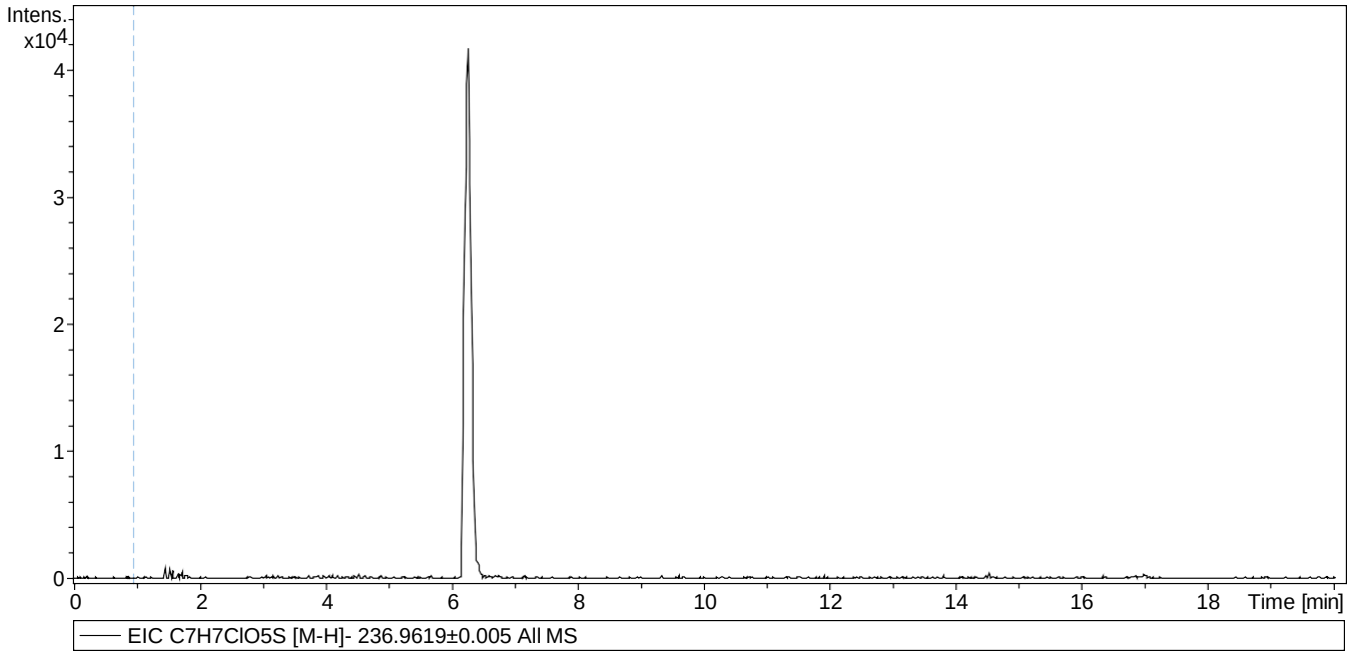
Acquisition Date 1/12/2016 8:13:51 PM

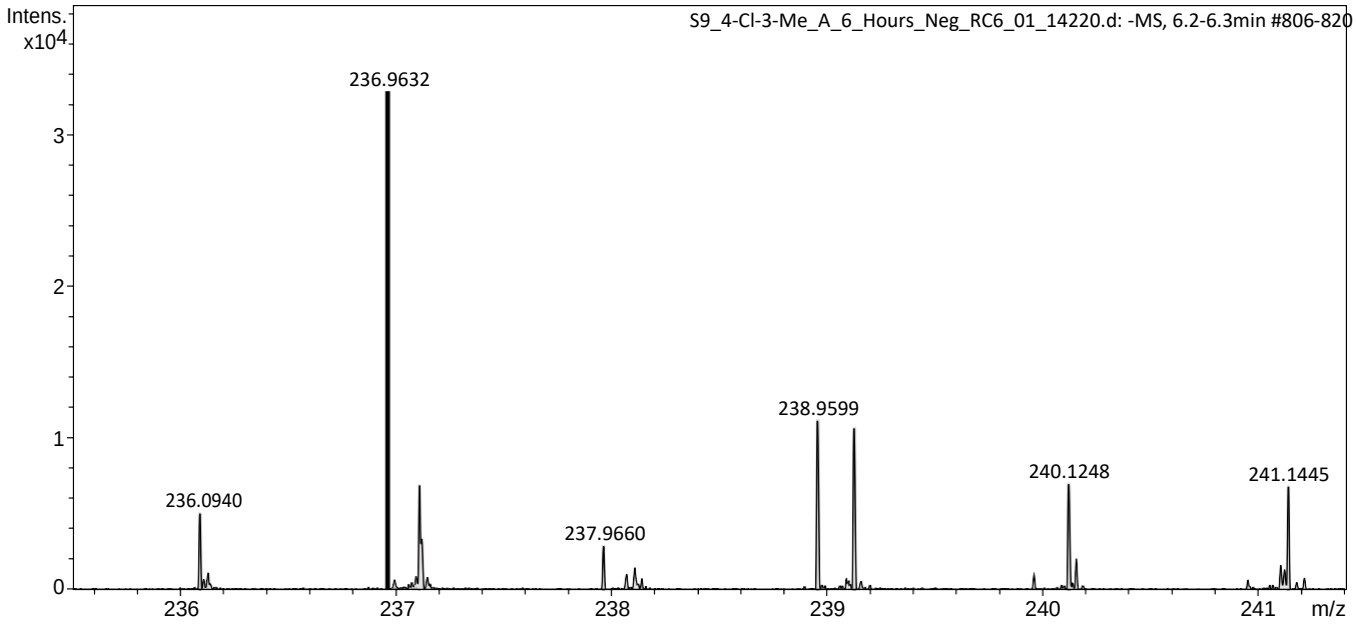
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

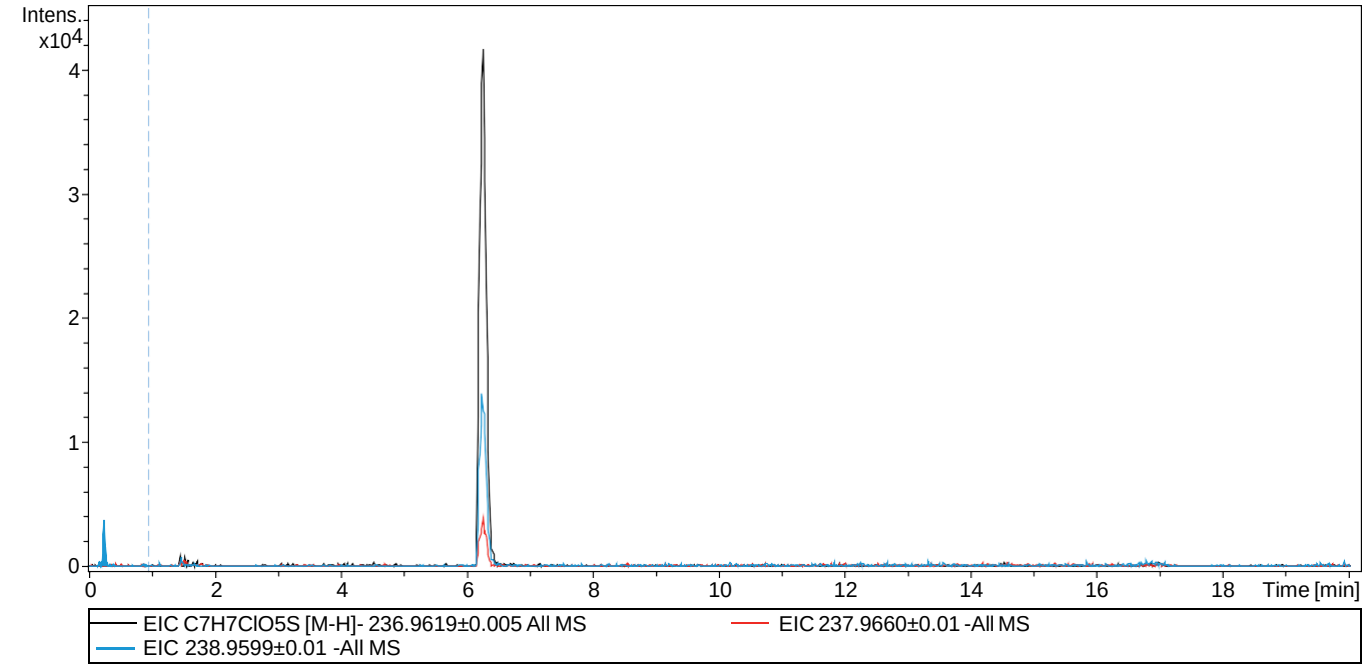
Acquisition Date 1/12/2016 8:13:51 PM

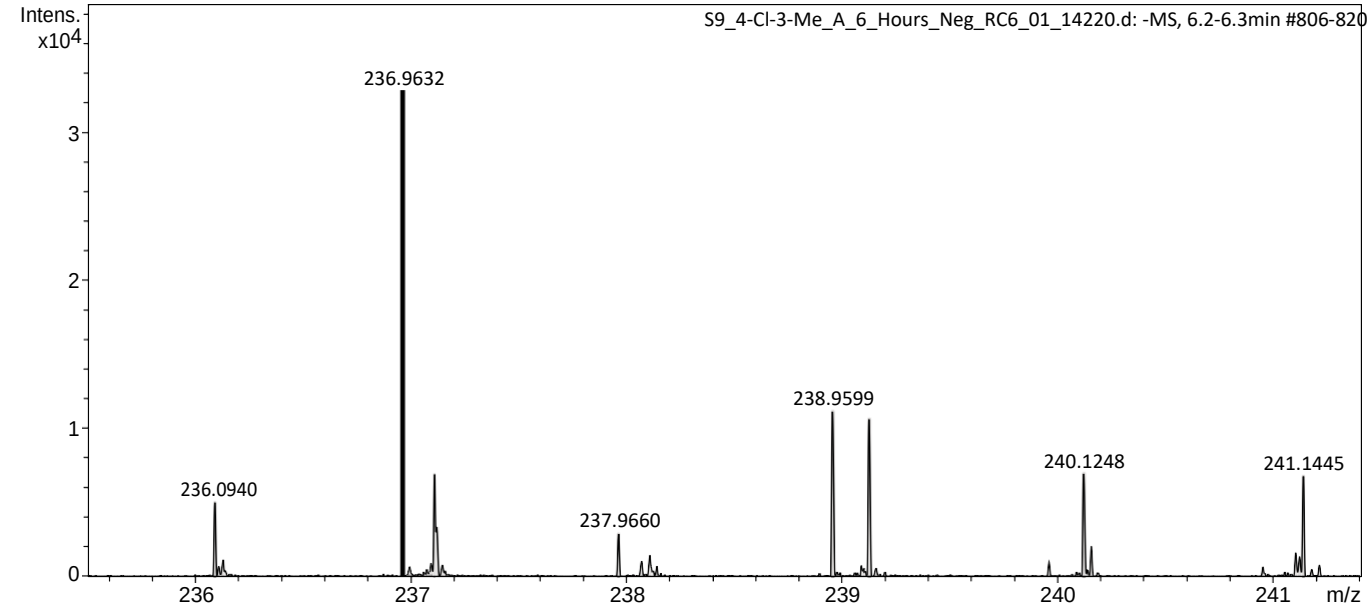
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

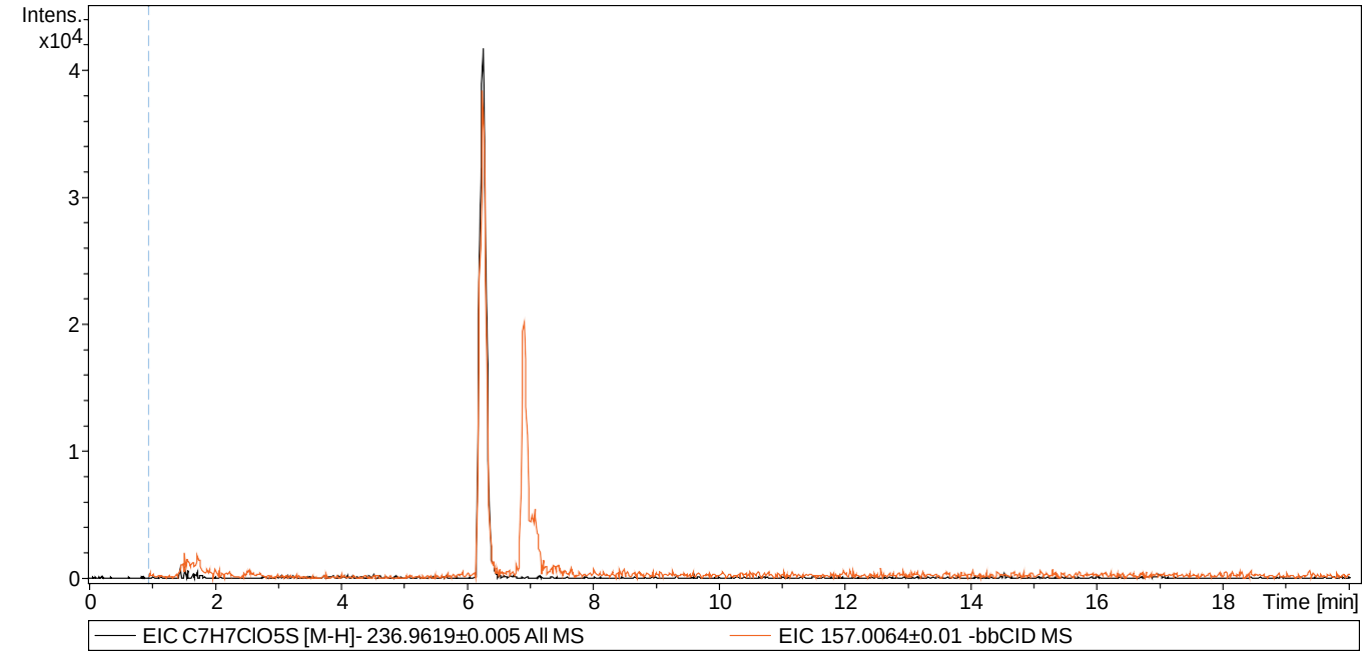
Acquisition Date 1/12/2016 8:13:51 PM

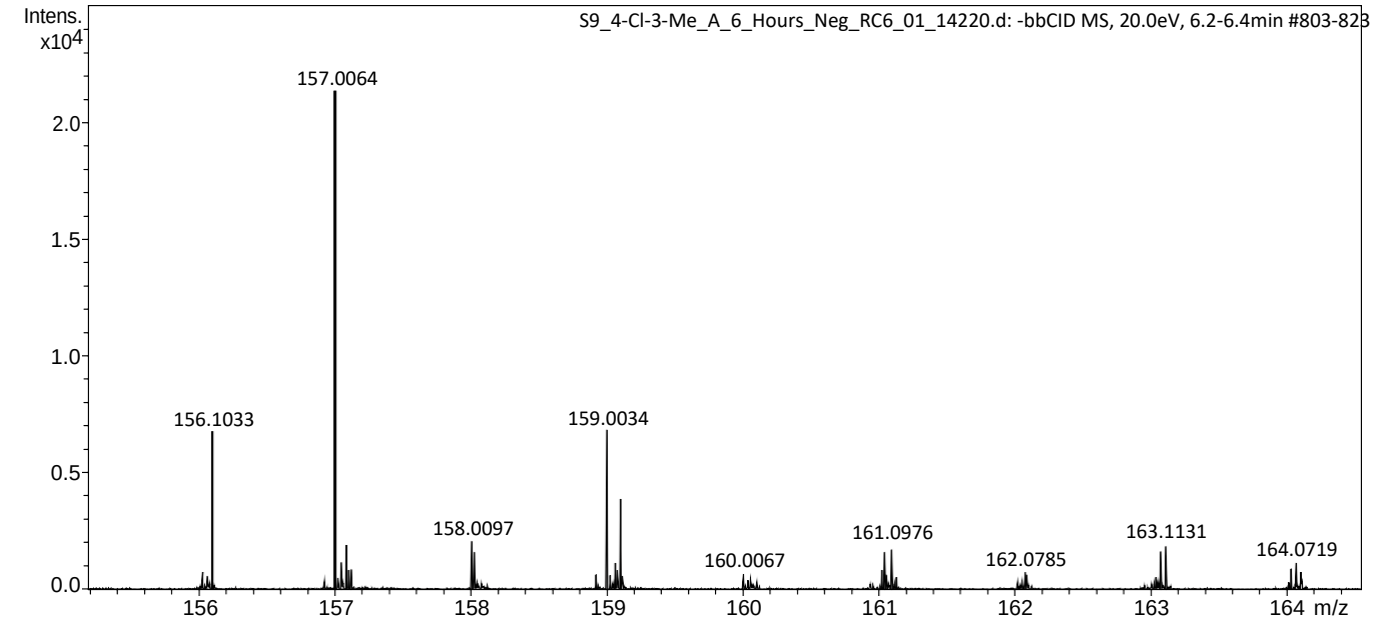
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

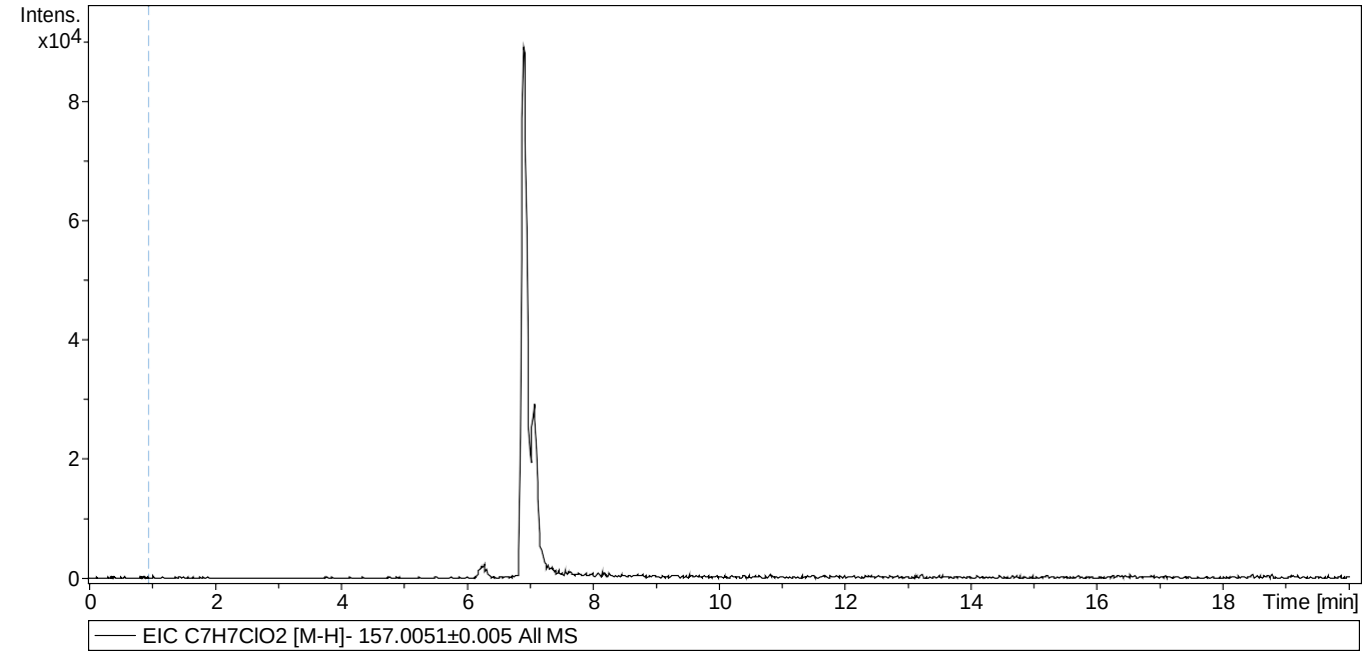
Acquisition Date 1/12/2016 10:21:32 PM

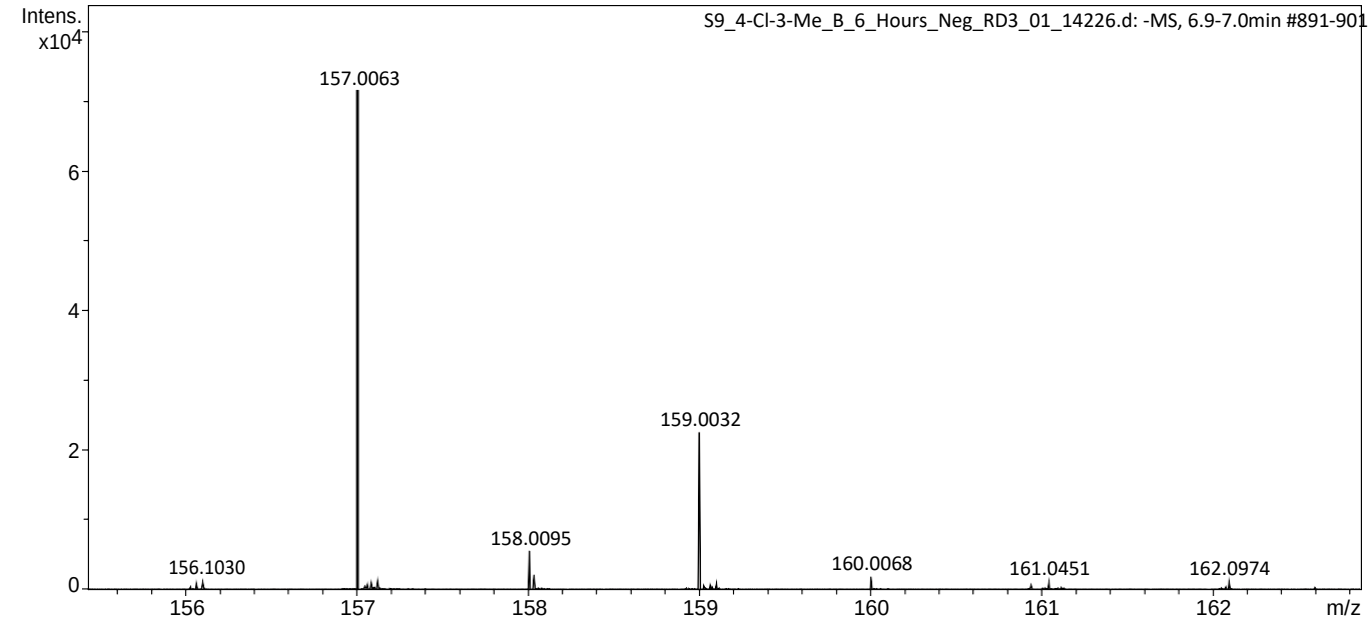
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

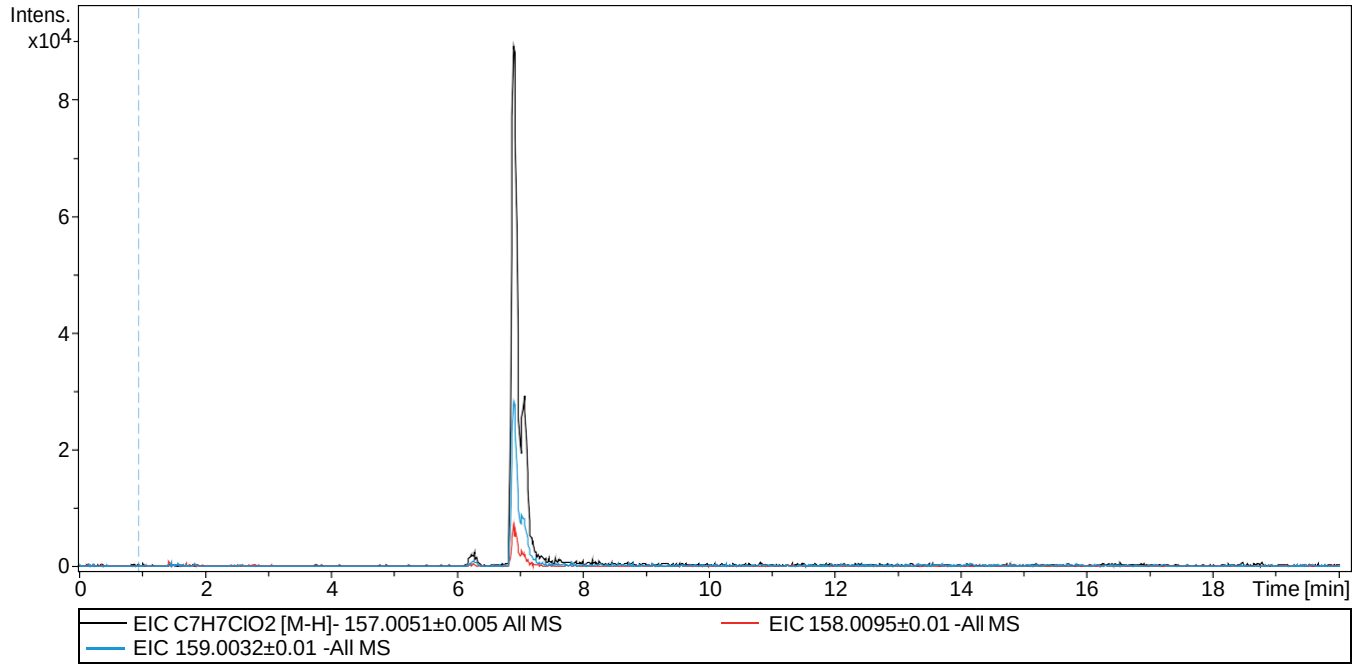
Acquisition Date 1/12/2016 10:21:32 PM

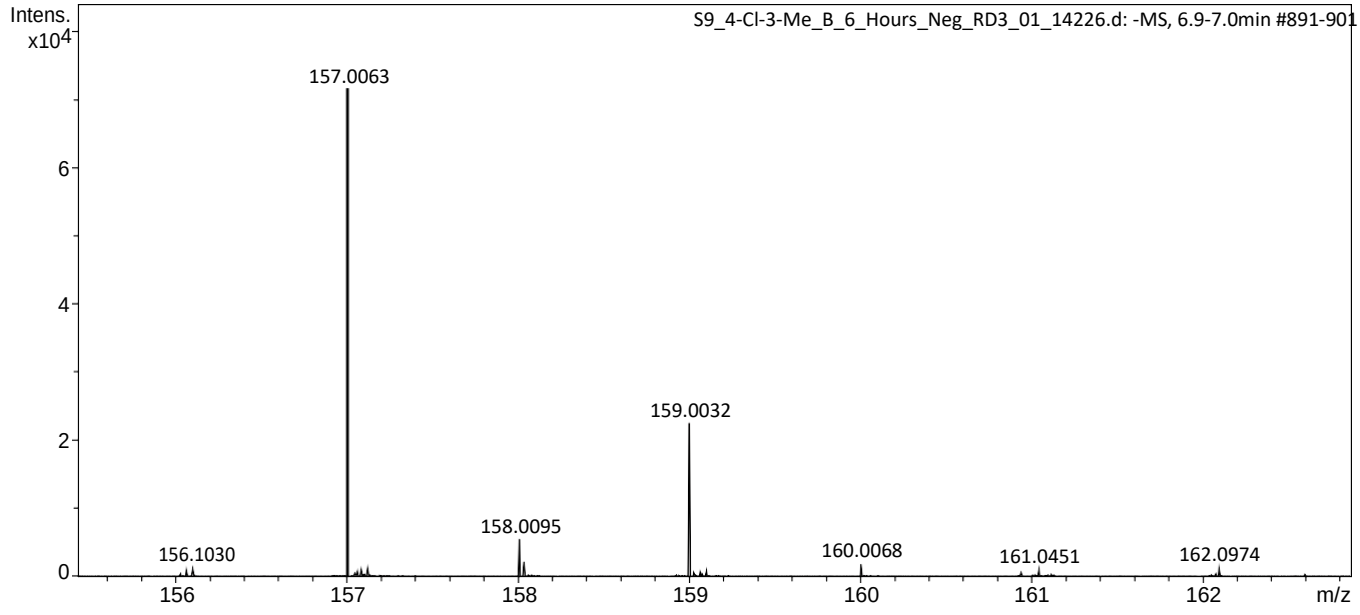
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 20:42:37

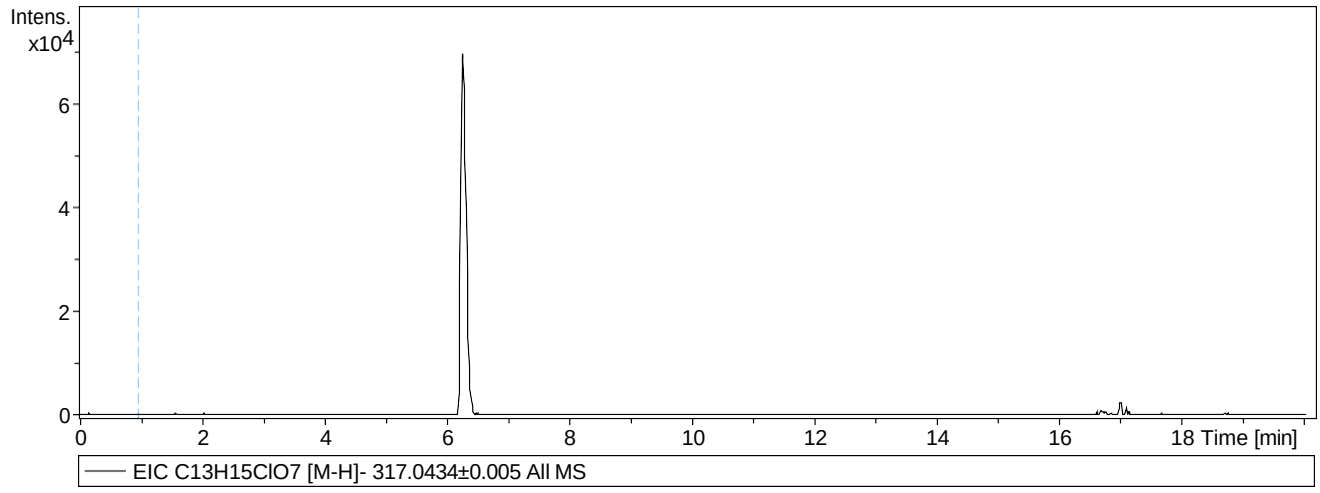
Operator BDAL@DE

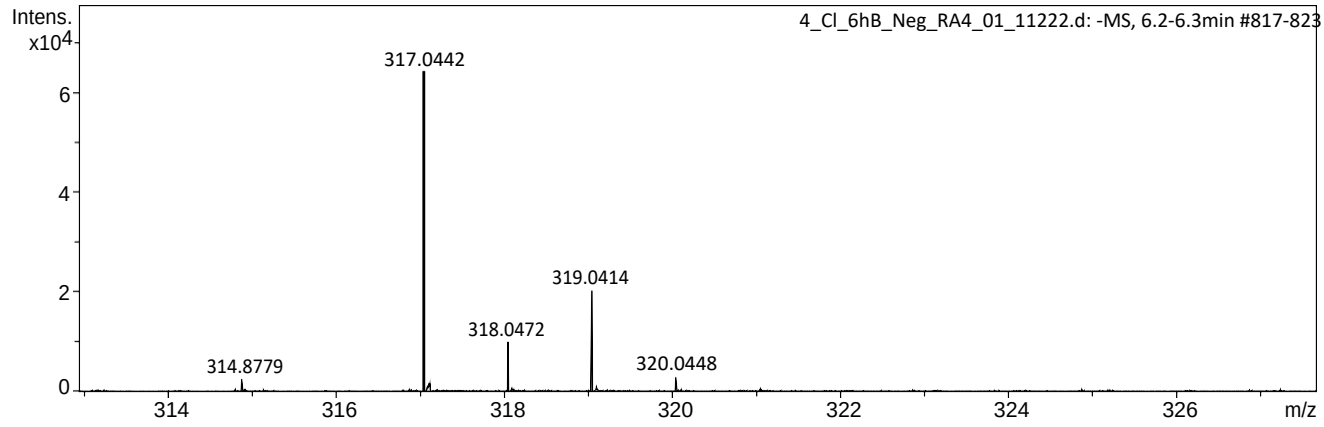
Sample Name 4_Cl_6hB_Neg

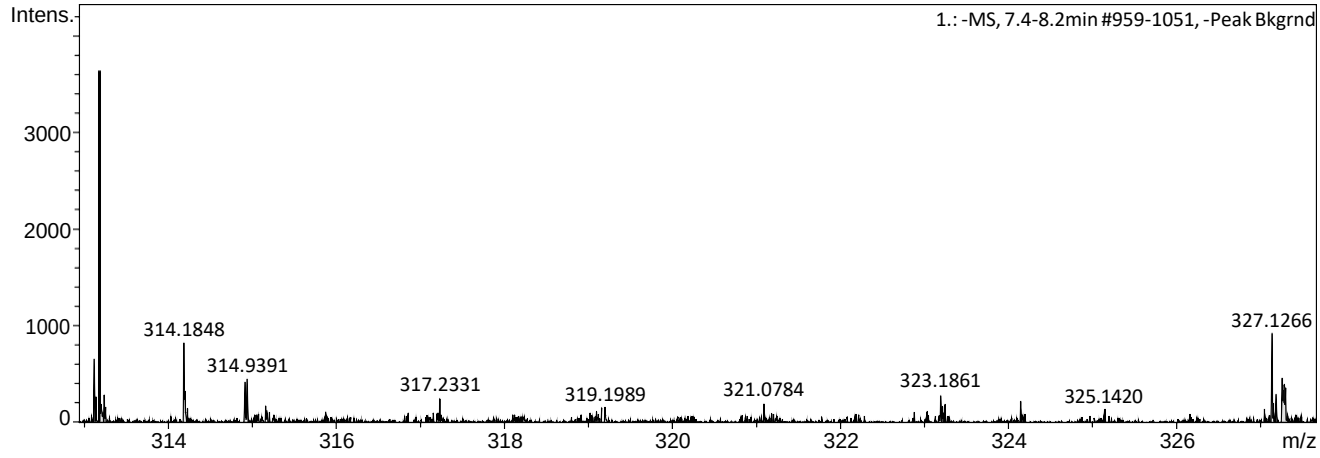
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C







Display Report

Analysis Info

Acquisition Date 15/03/2017 20:42:37

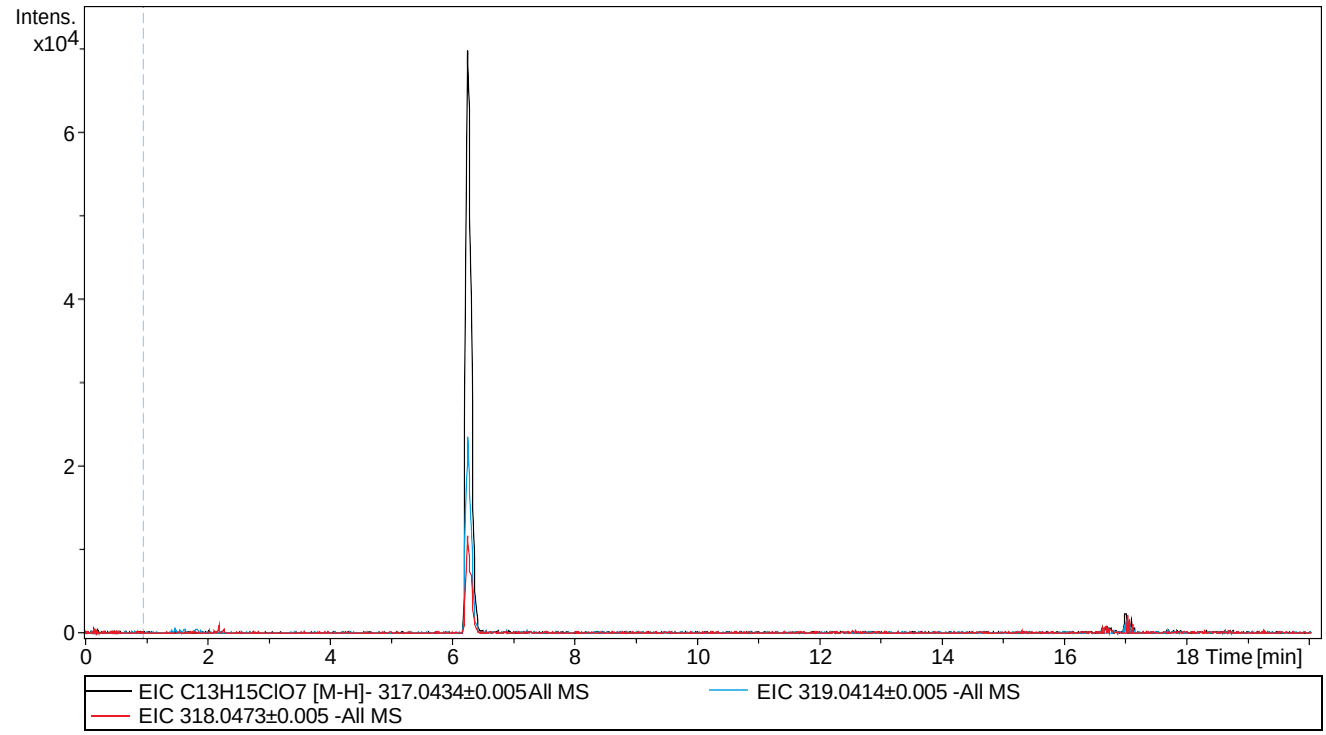
Operator BDAL@DE

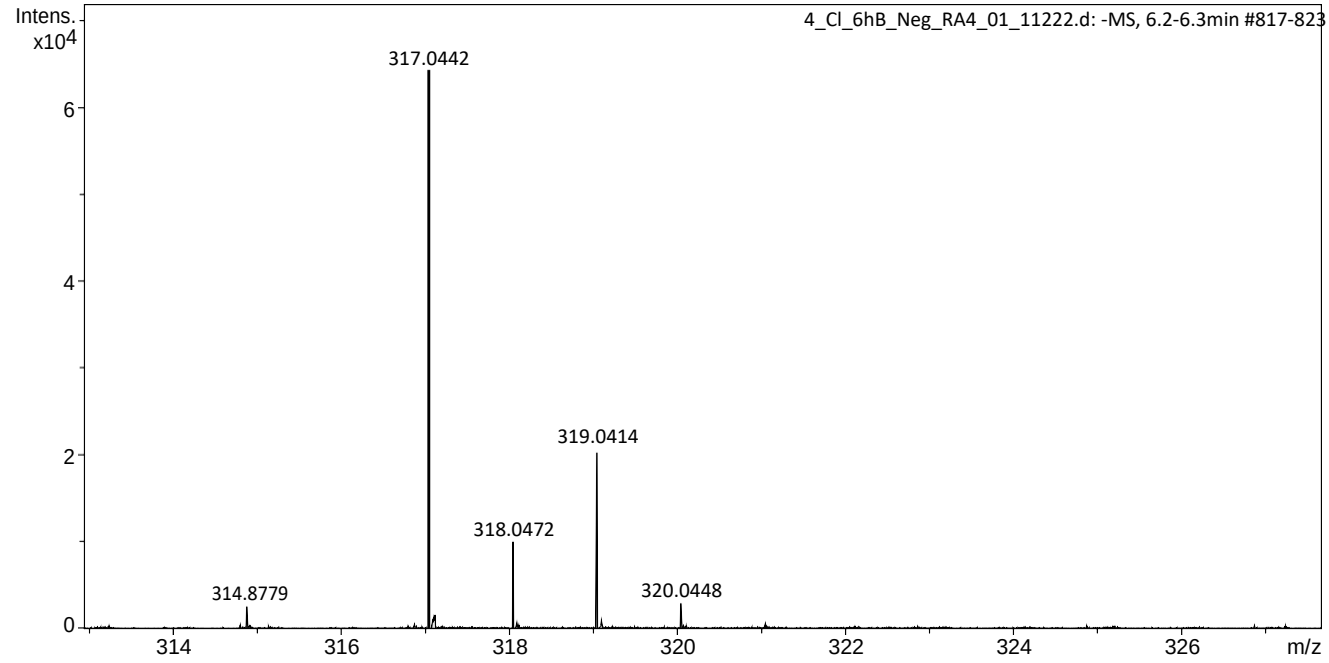
Sample Name 4_Cl_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 20:42:37

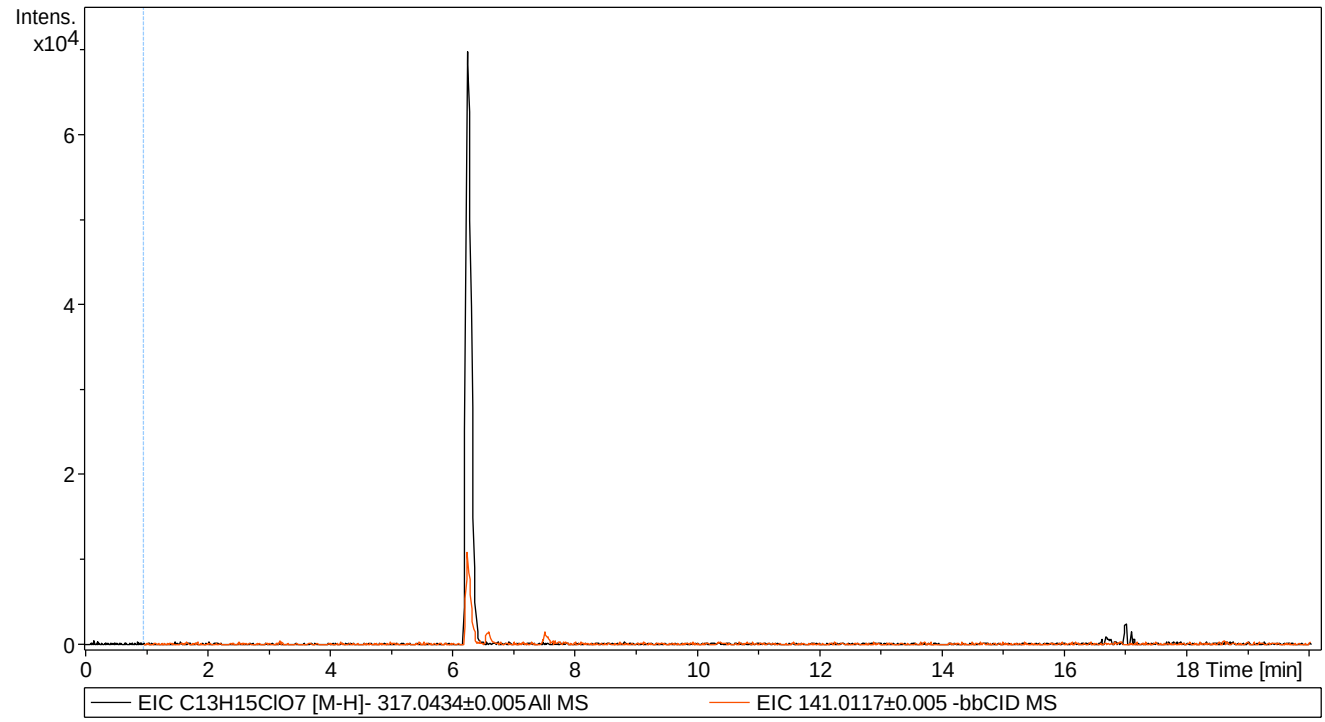
Operator BDAL@DE

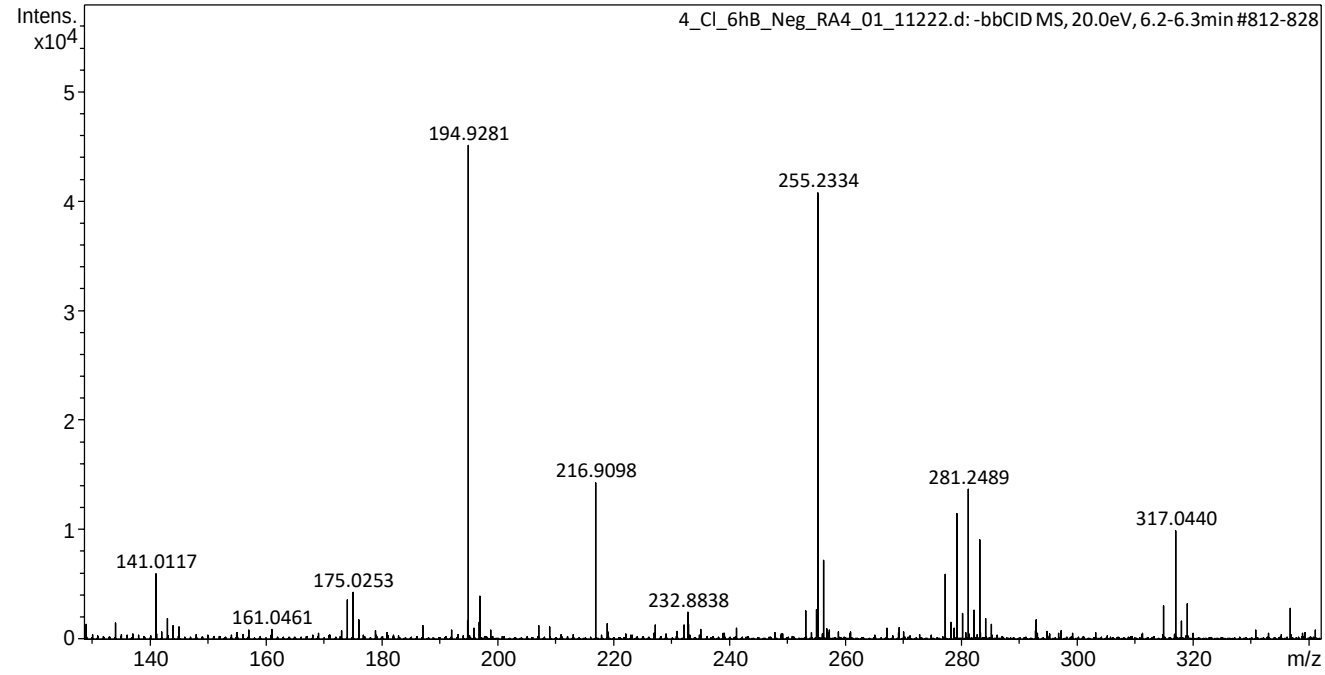
Sample Name 4_Cl_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





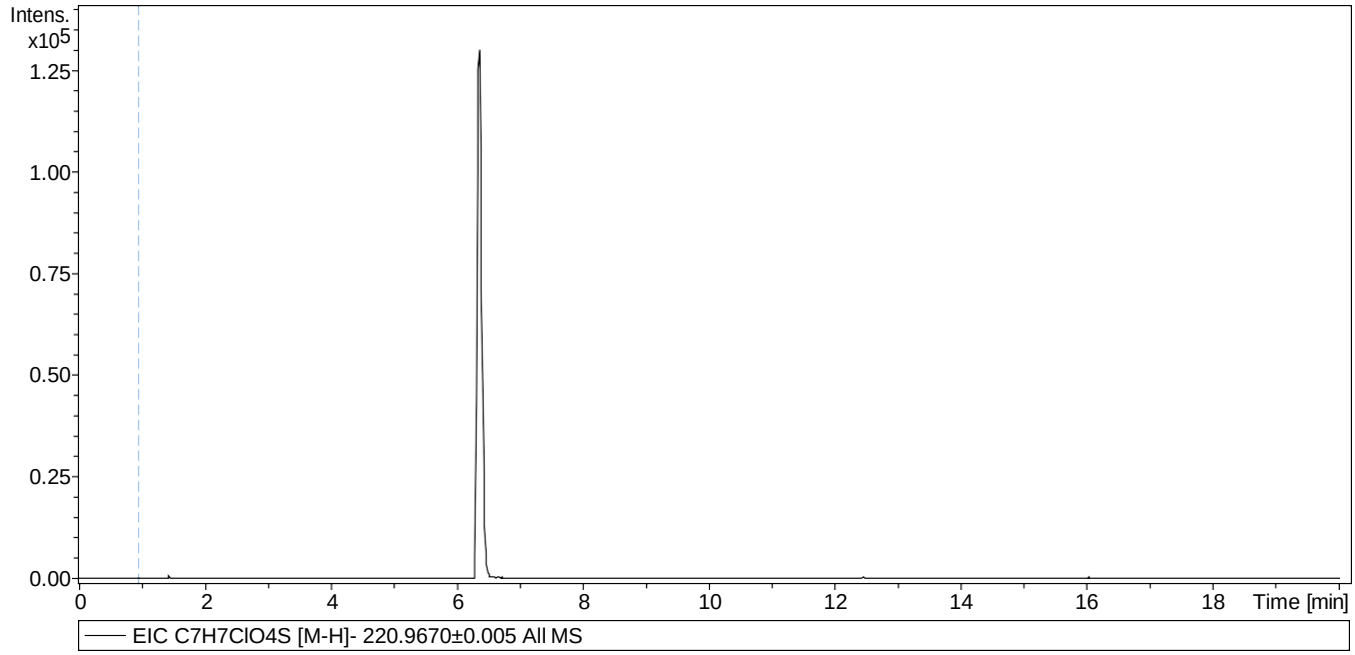
Display Report

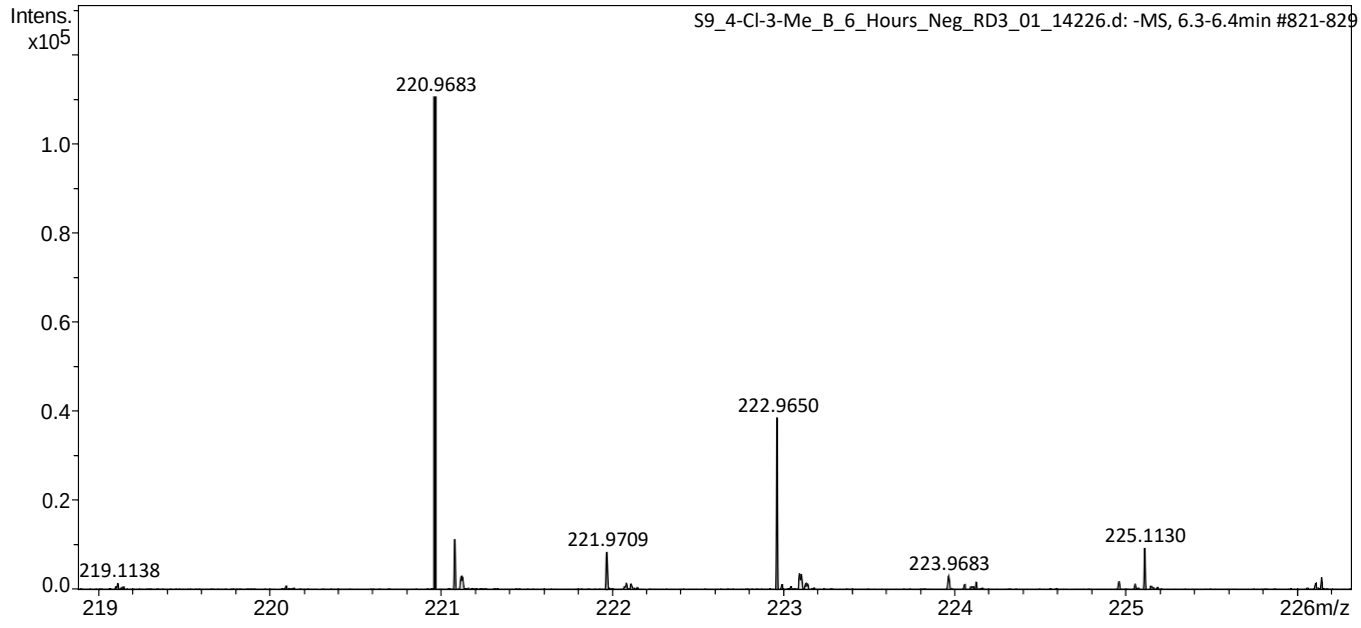
Analysis Info

Acquisition Date	1/12/2016 10:21:32 PM		
Sample Name	S9_4-Cl-3-Me_B_6_Hours_Neg	Operator	CCAF
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

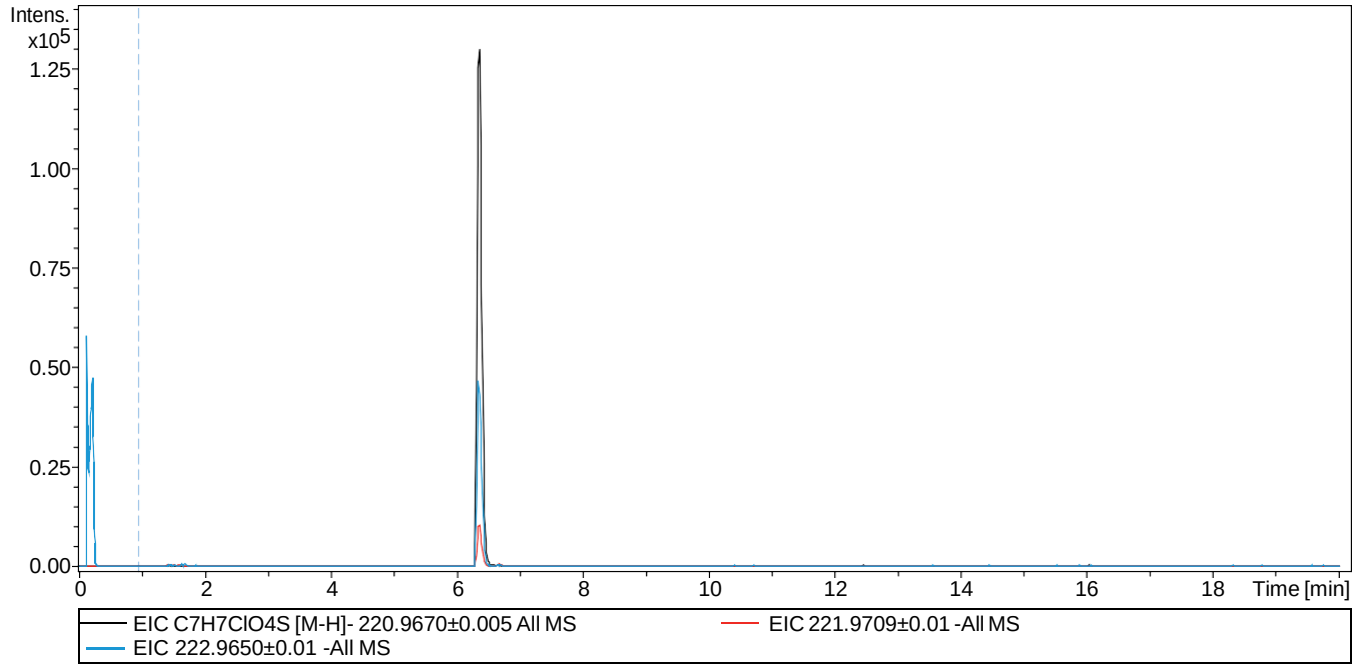
Acquisition Date 1/12/2016 10:21:32 PM

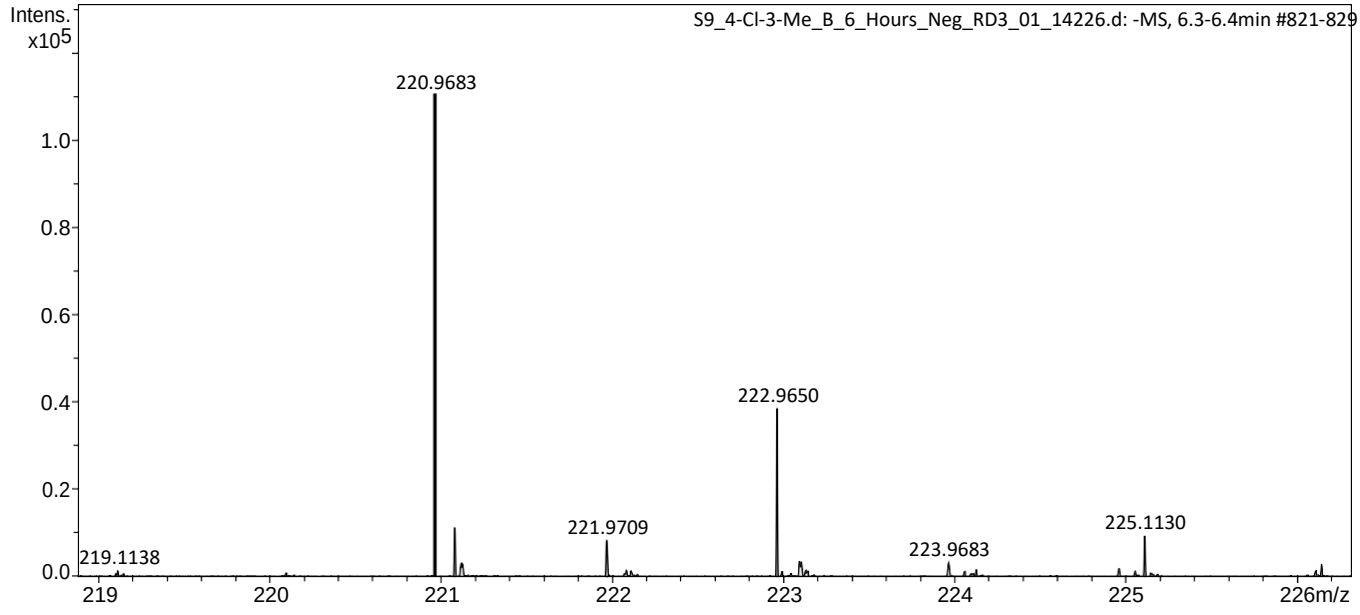
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

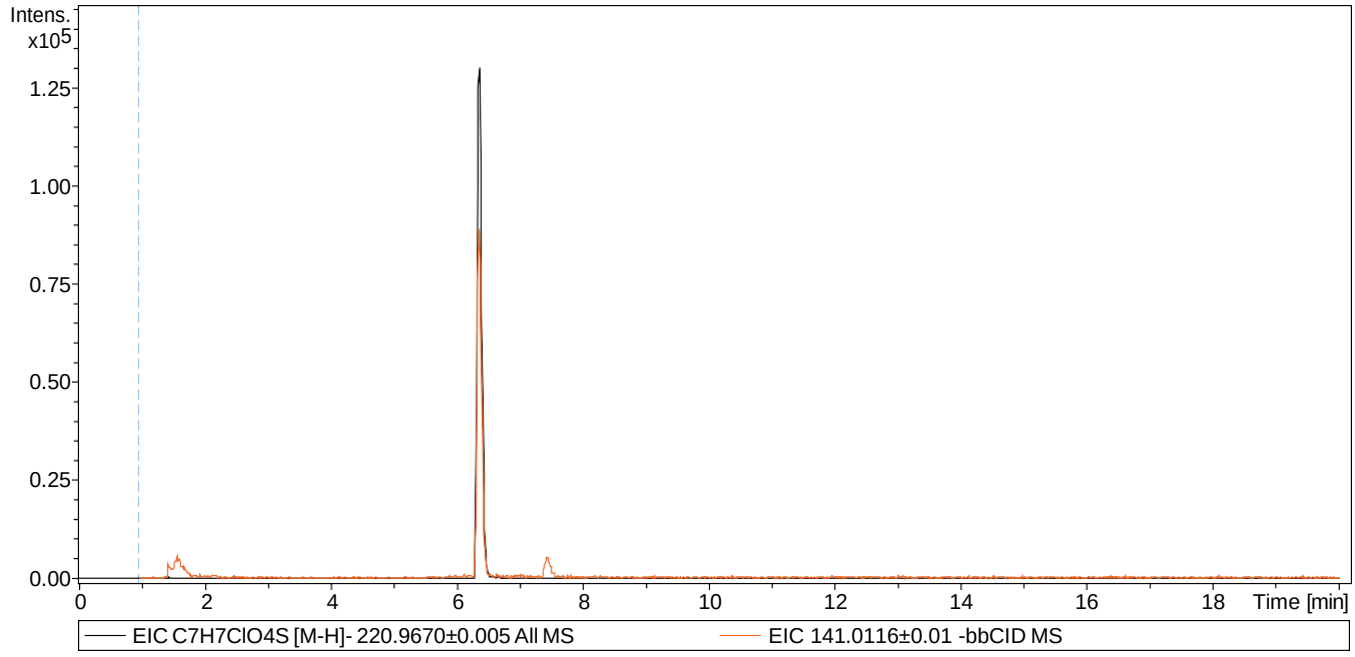
Acquisition Date 1/12/2016 10:21:32 PM

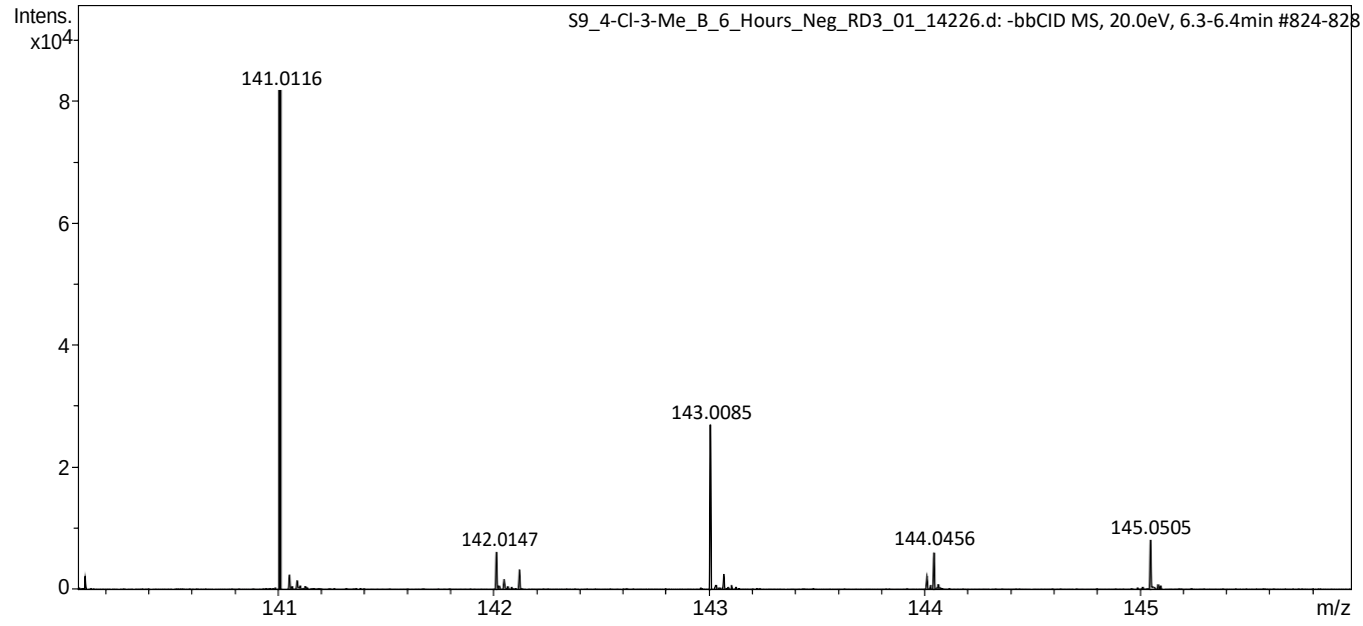
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

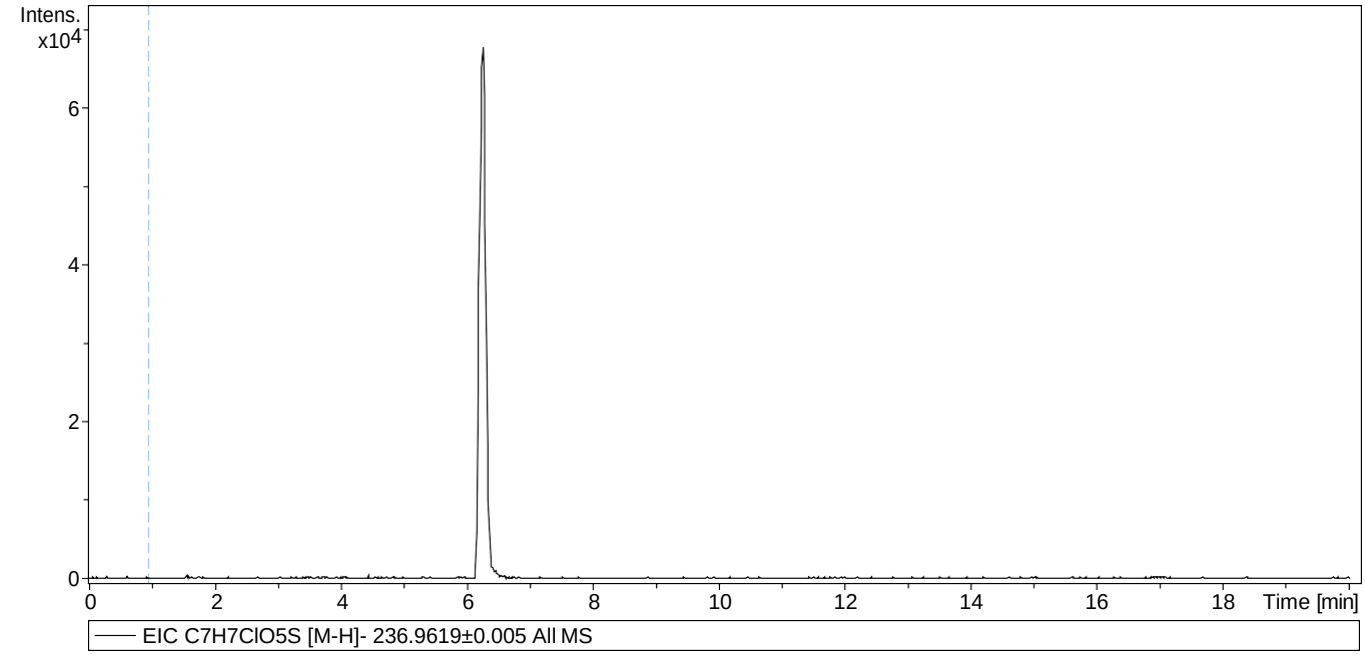
Acquisition Date 1/12/2016 10:21:32 PM

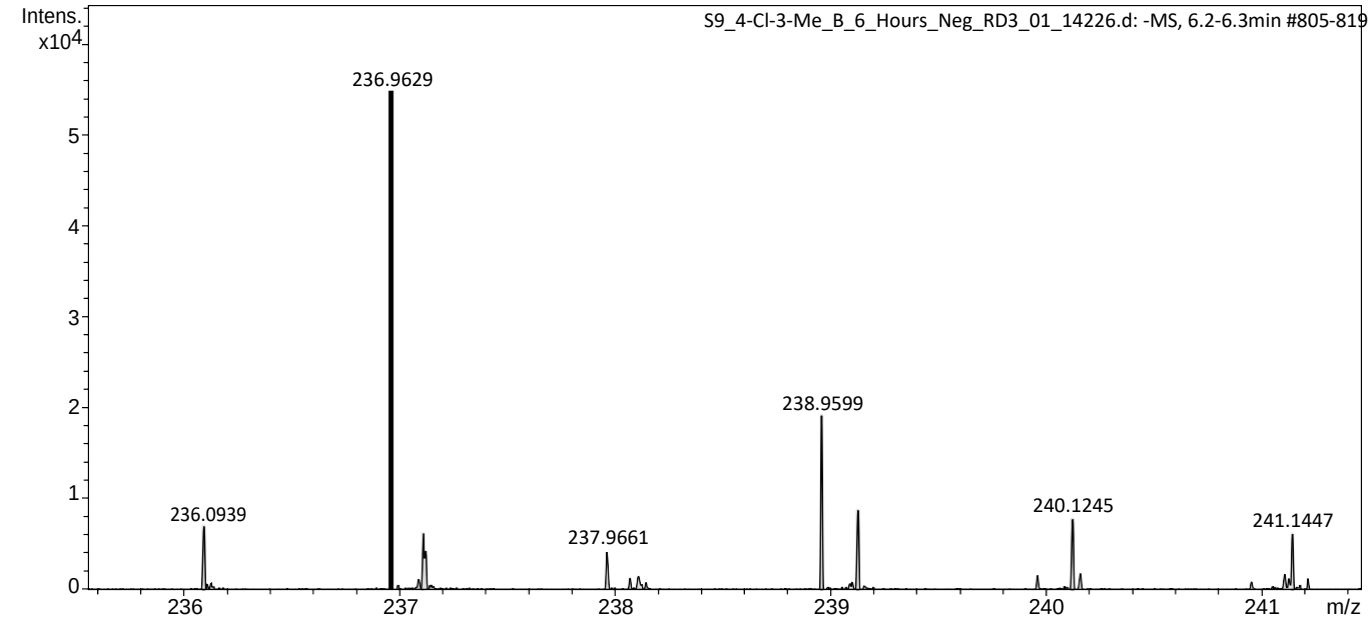
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

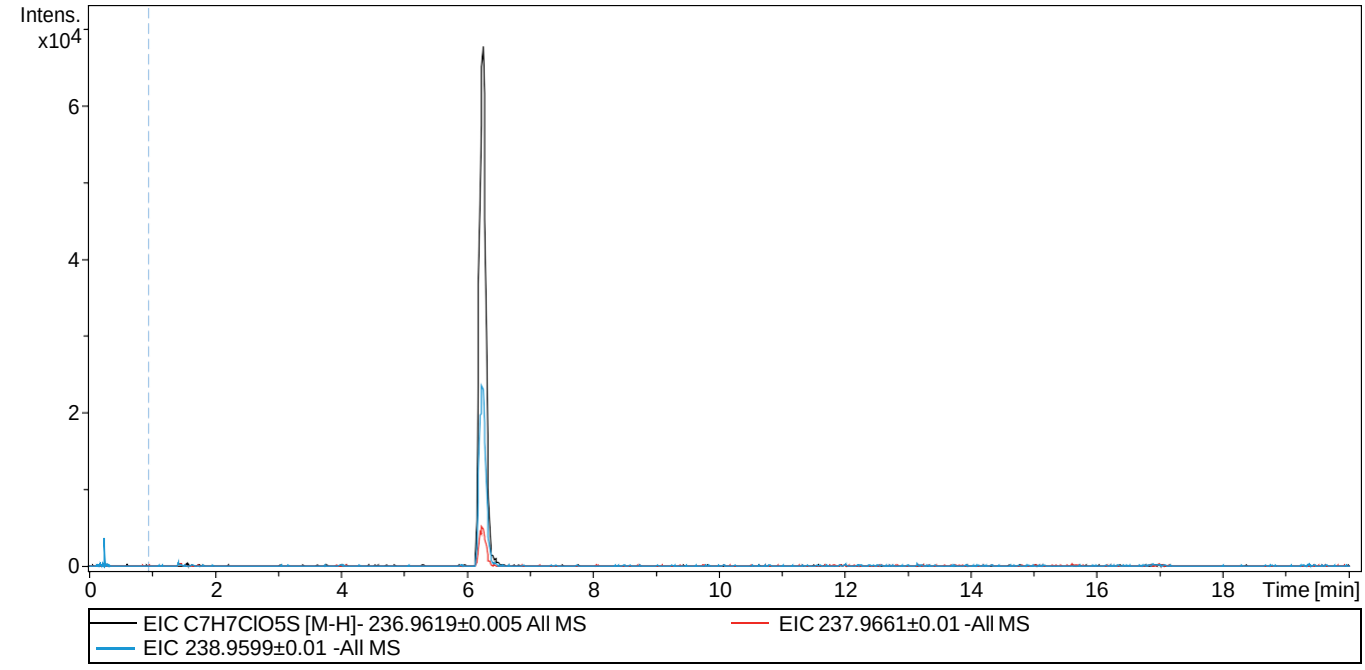
Acquisition Date 1/12/2016 10:21:32 PM

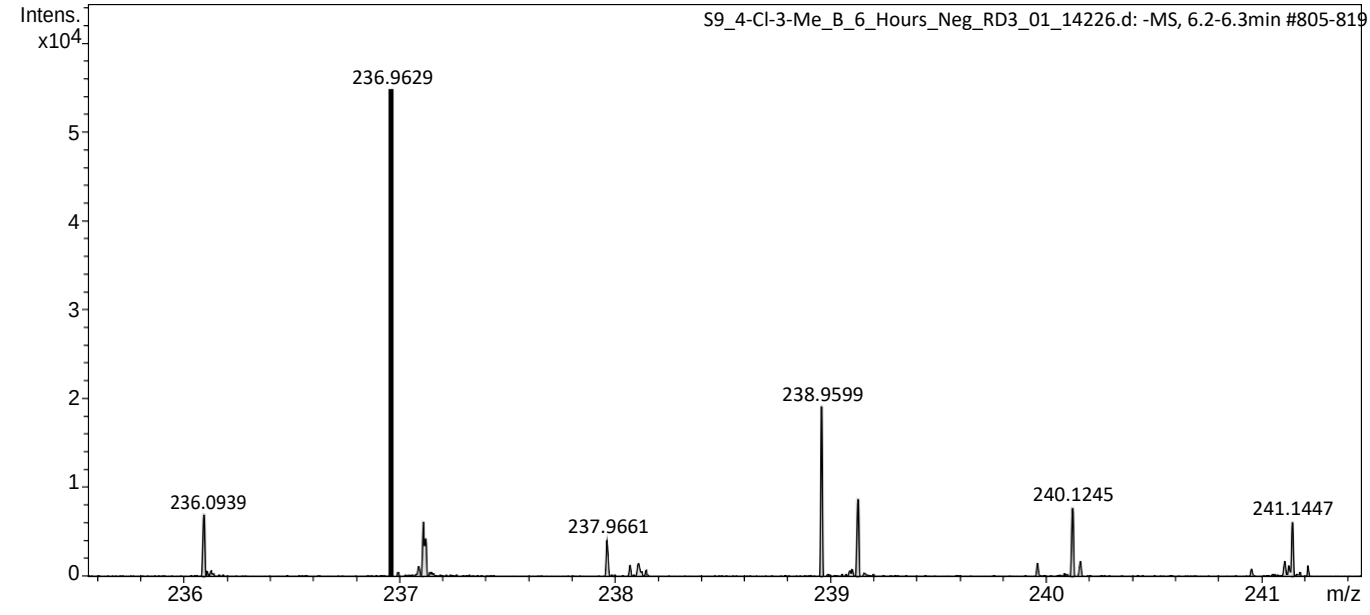
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

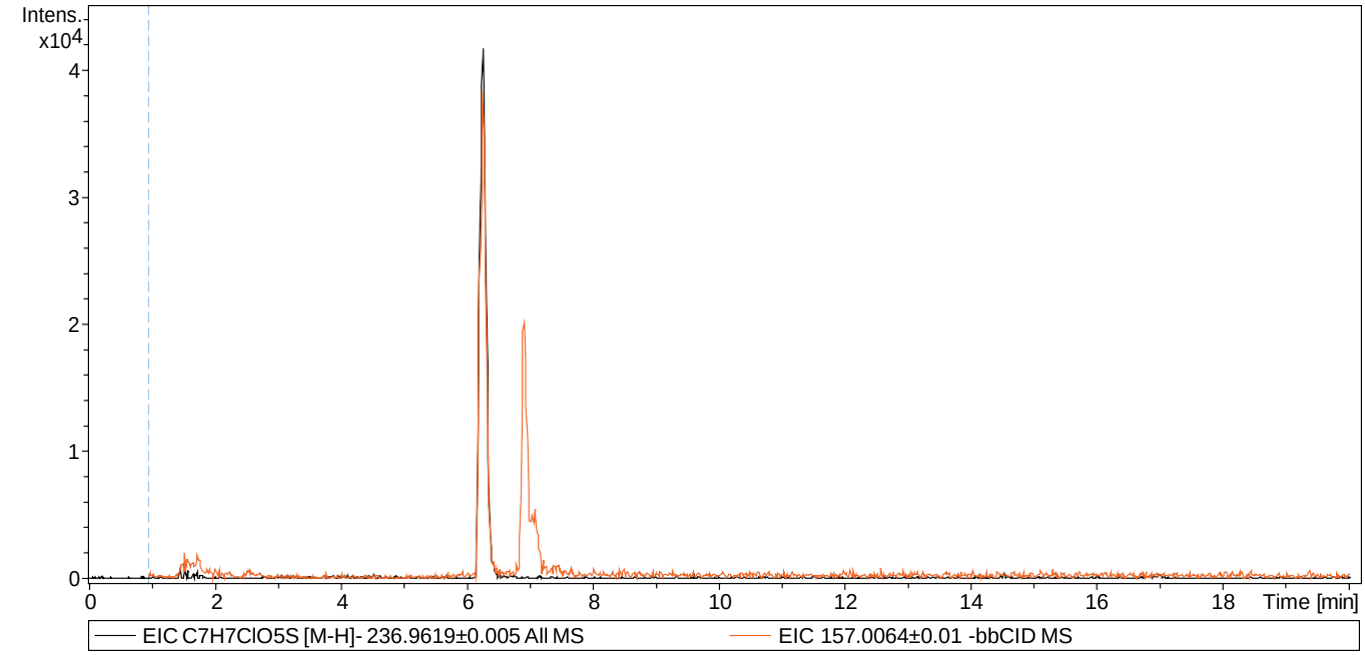
Acquisition Date 1/12/2016 8:13:51 PM

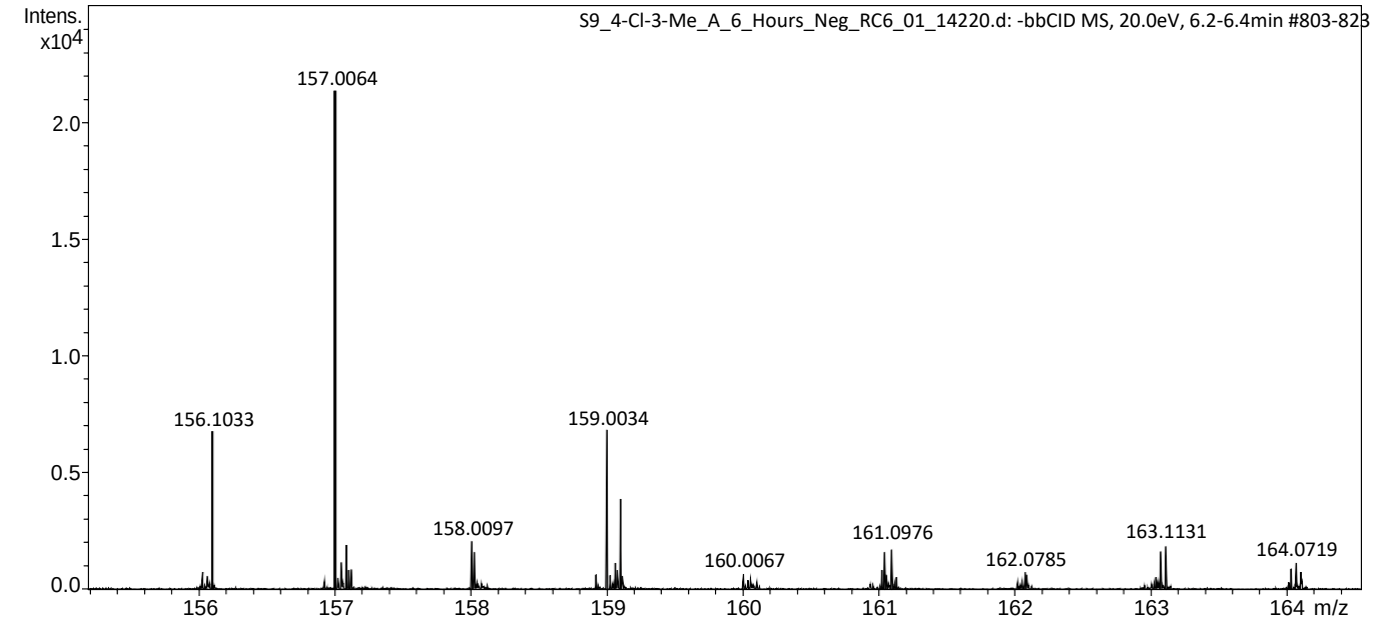
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

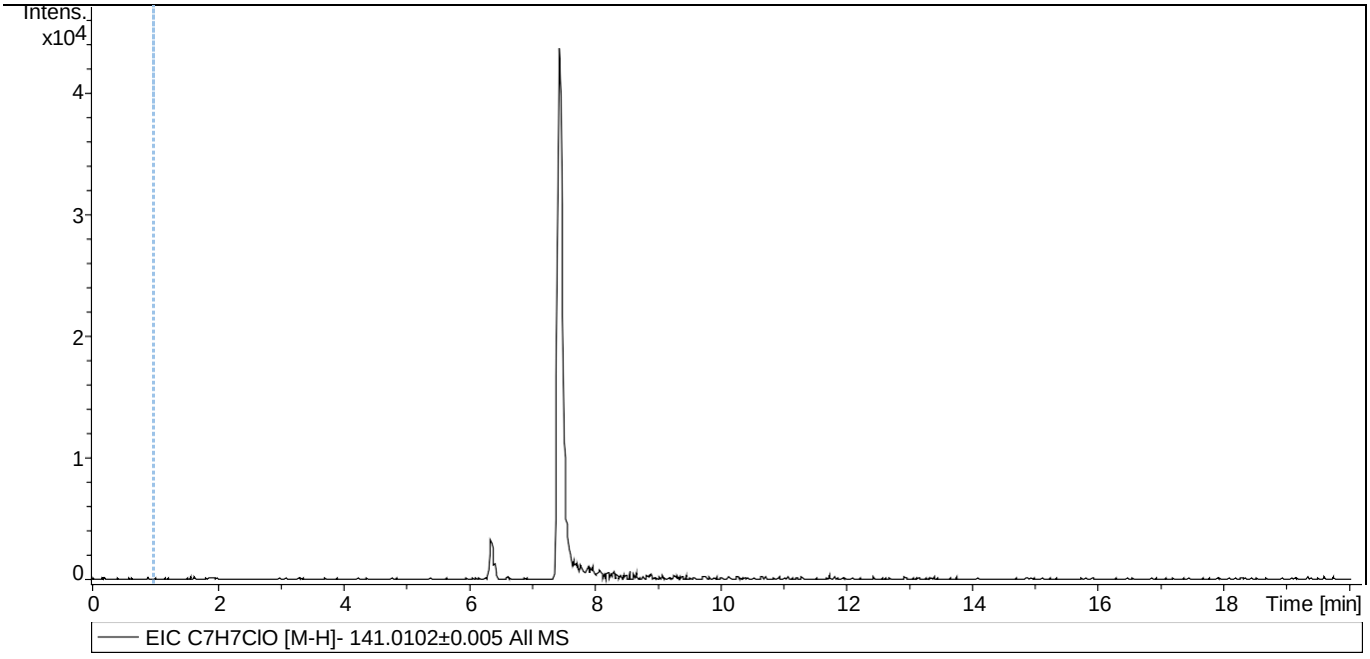
Acquisition Date 1/12/2016 8:13:51 PM

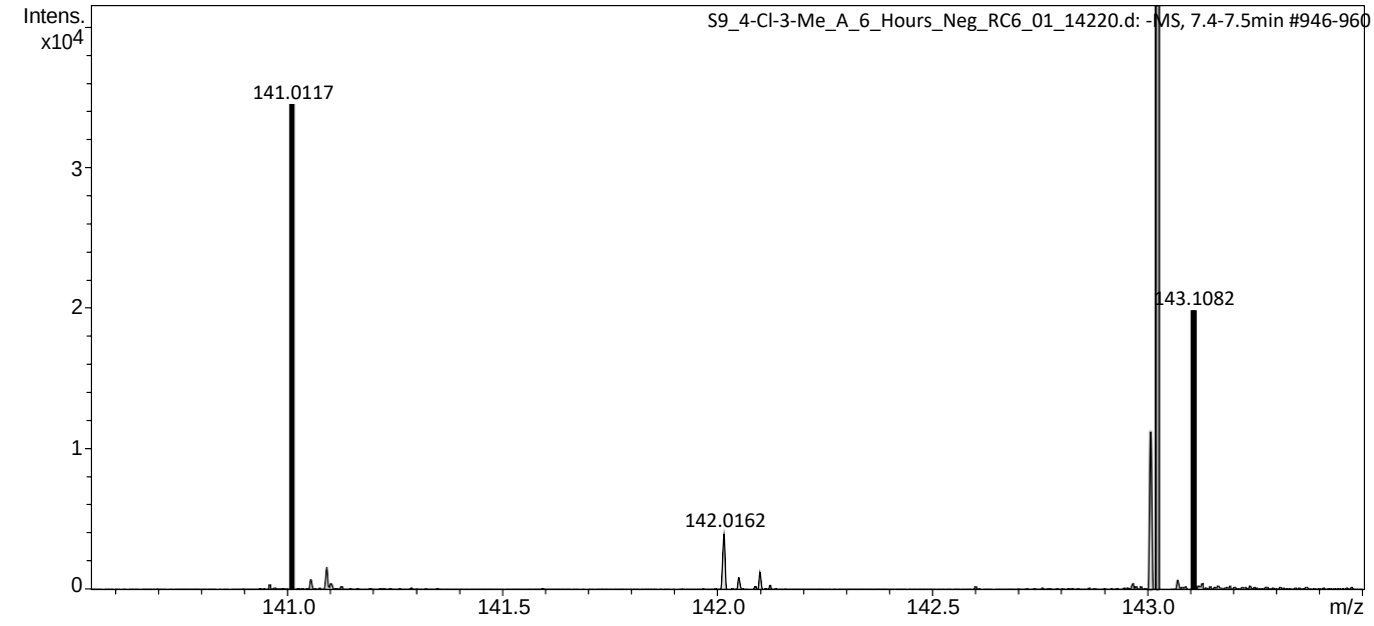
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

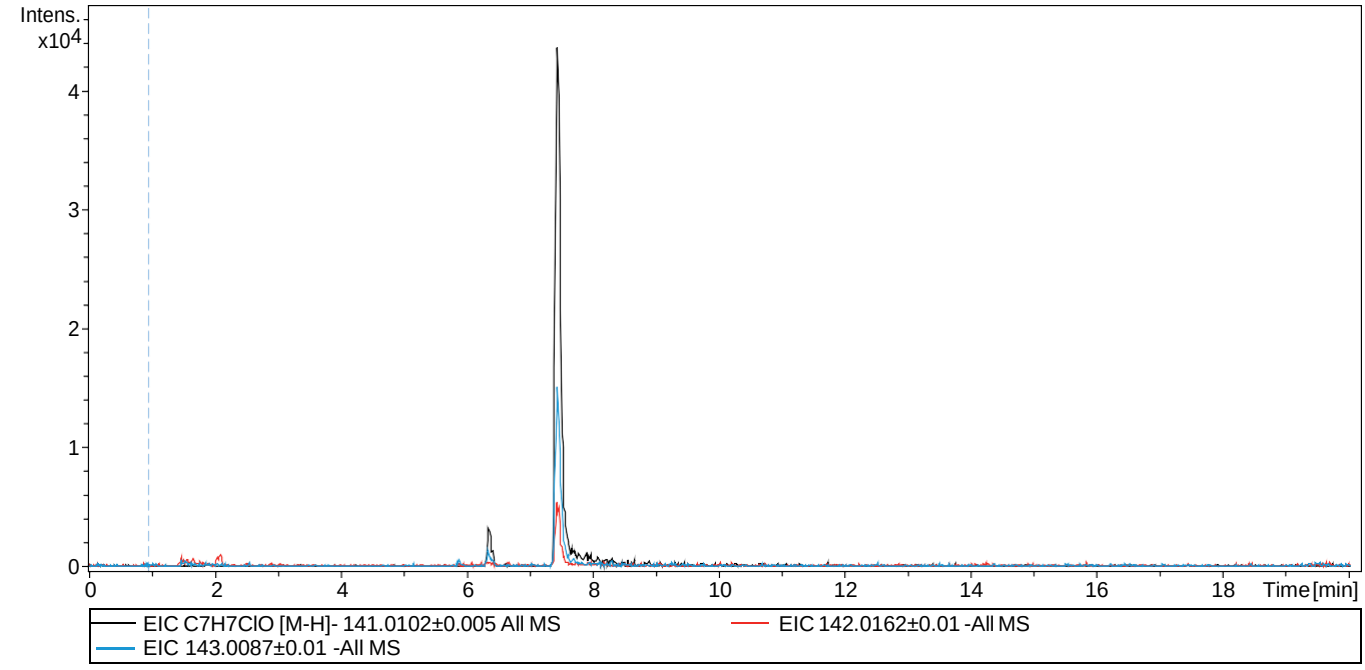
Acquisition Date 1/12/2016 8:13:51 PM

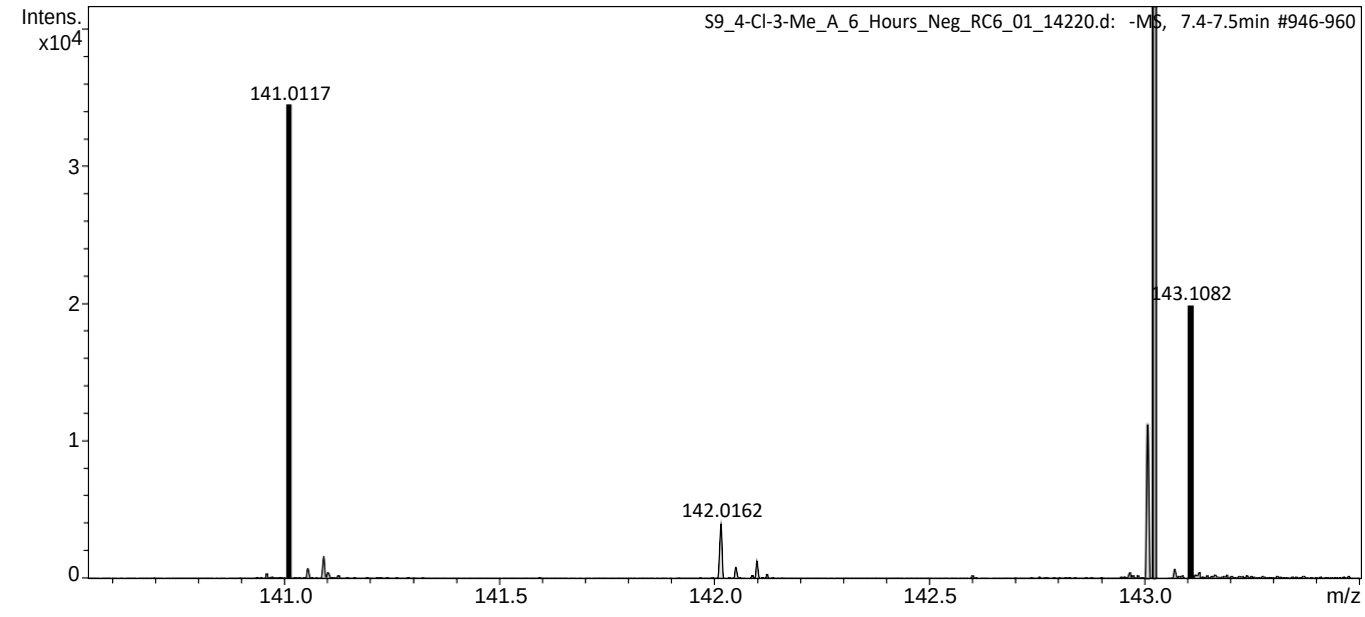
Sample Name S9_4-Cl-3-Me_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_A_6_Hours_Neg_RC6_01_14220.d

Display Report

Analysis Info

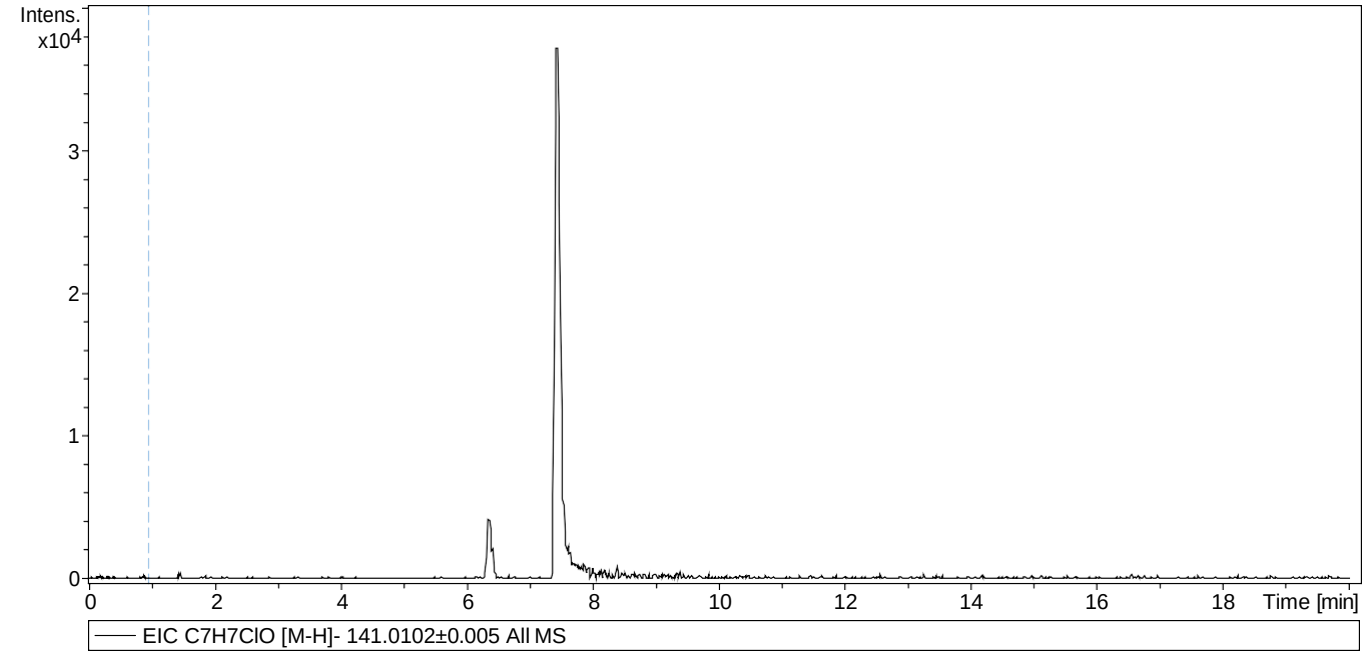
Acquisition Date 1/12/2016 10:21:32 PM

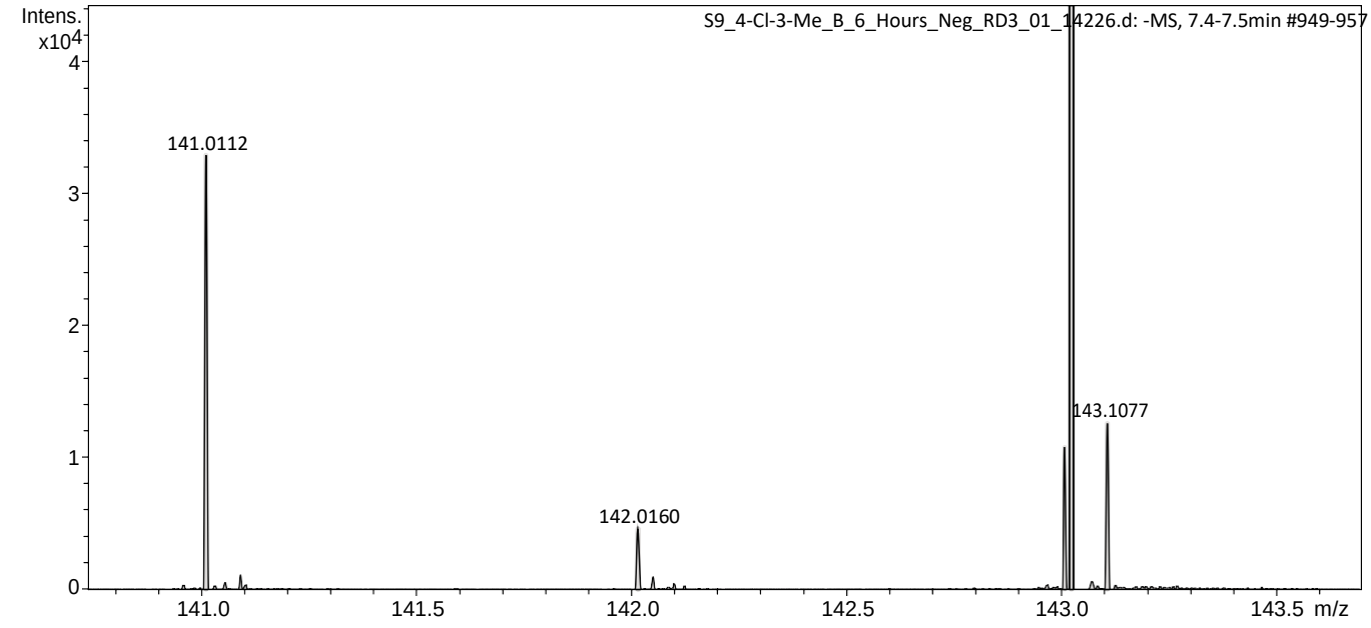
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

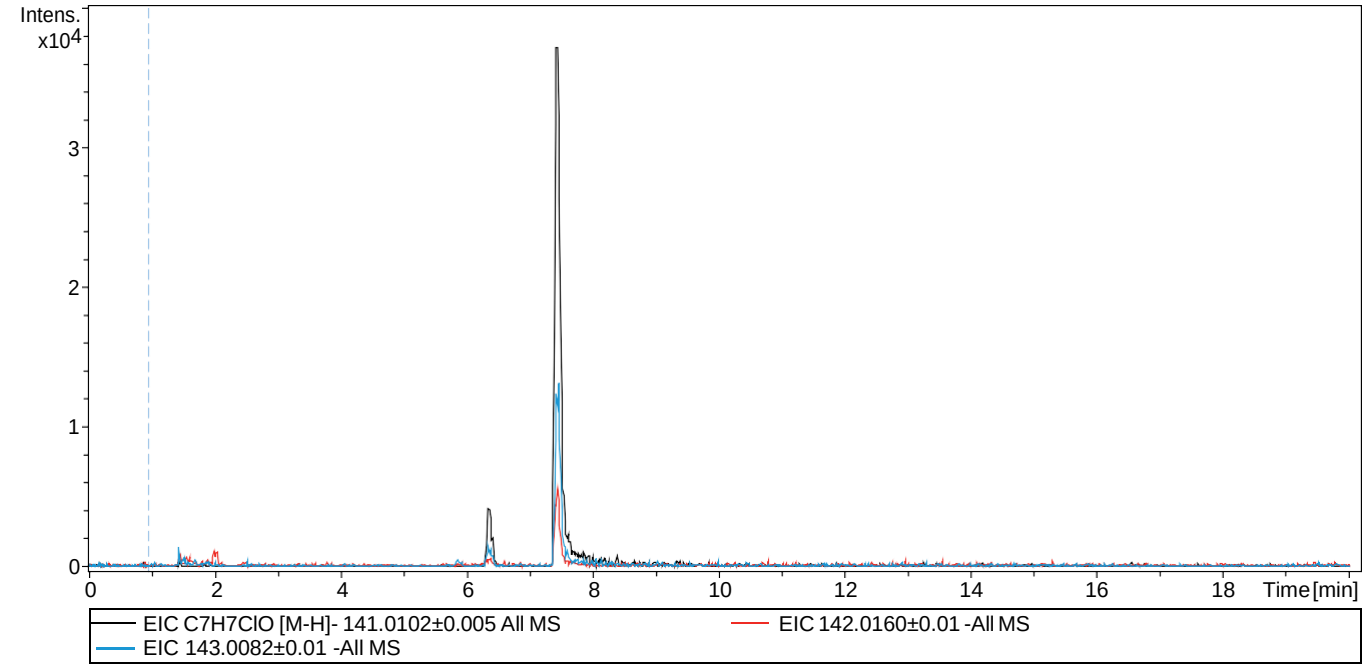
Acquisition Date 1/12/2016 10:21:32 PM

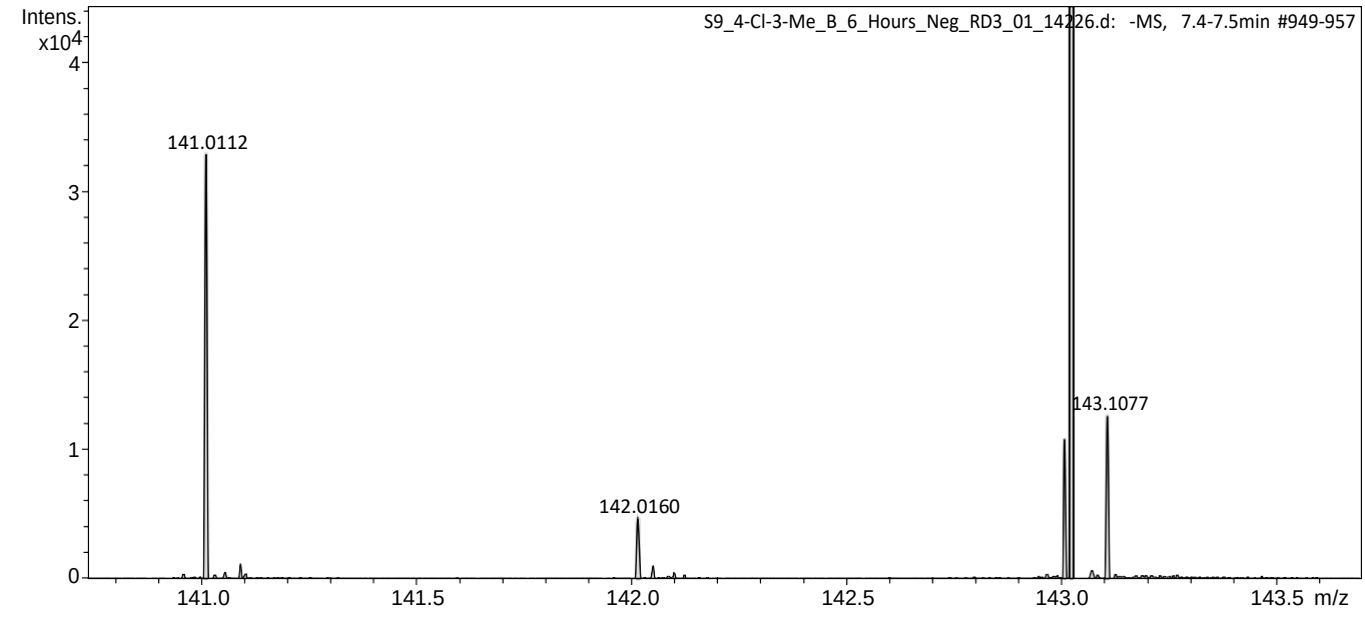
Sample Name S9_4-Cl-3-Me_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_B_6_Hours_Neg_RD3_01_14226.d

Display Report

Analysis Info

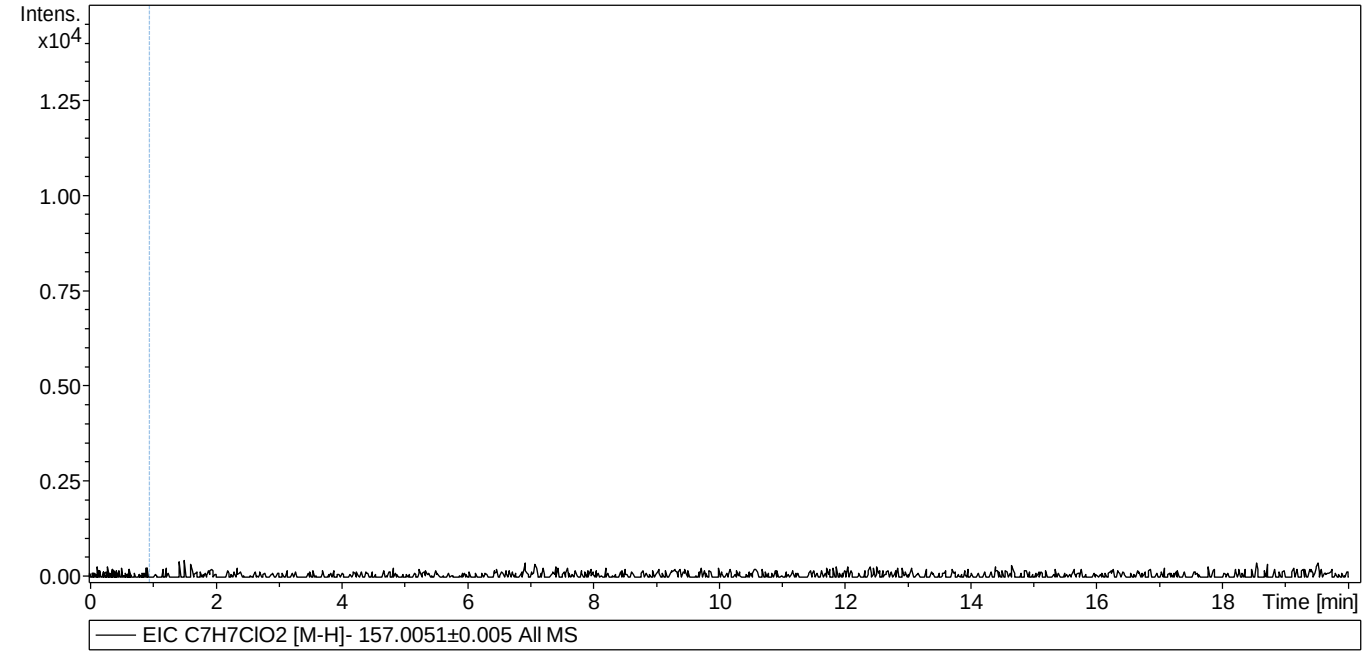
Acquisition Date 1/12/2016 5:23:29 PM

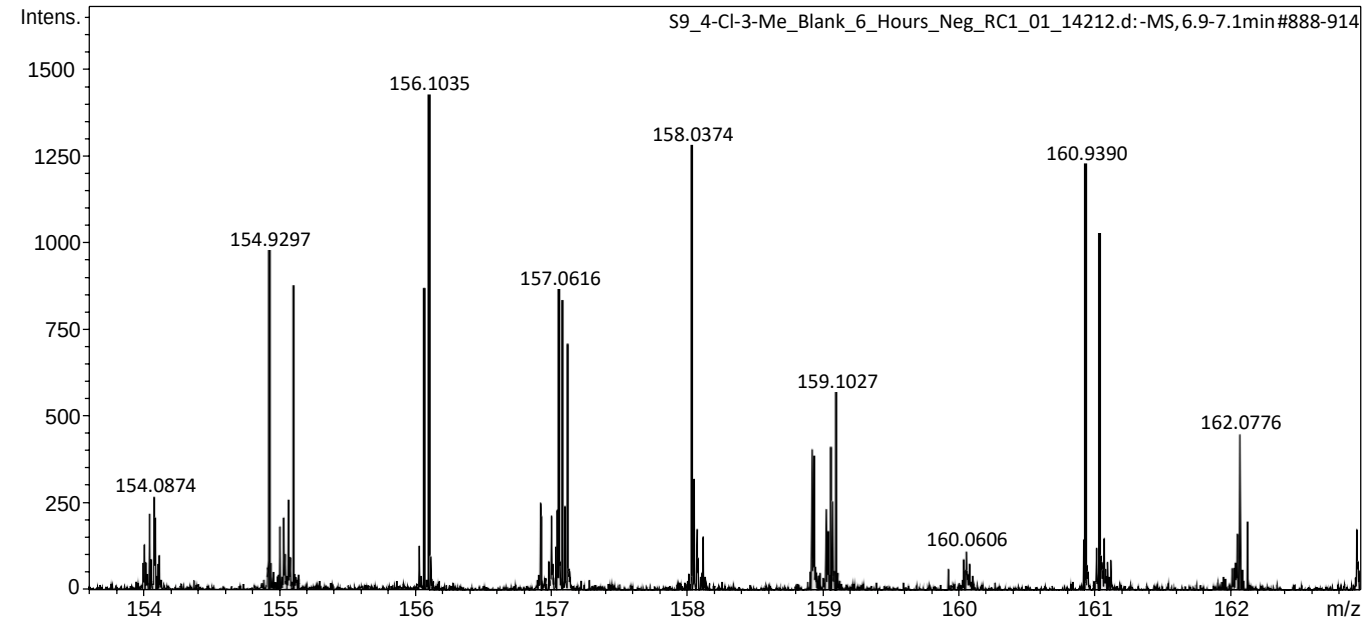
Sample Name S9_4-Cl-3-Me_Blank_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_Blank_6_Hours_Neg_RC1_01_14212.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 21:03:52

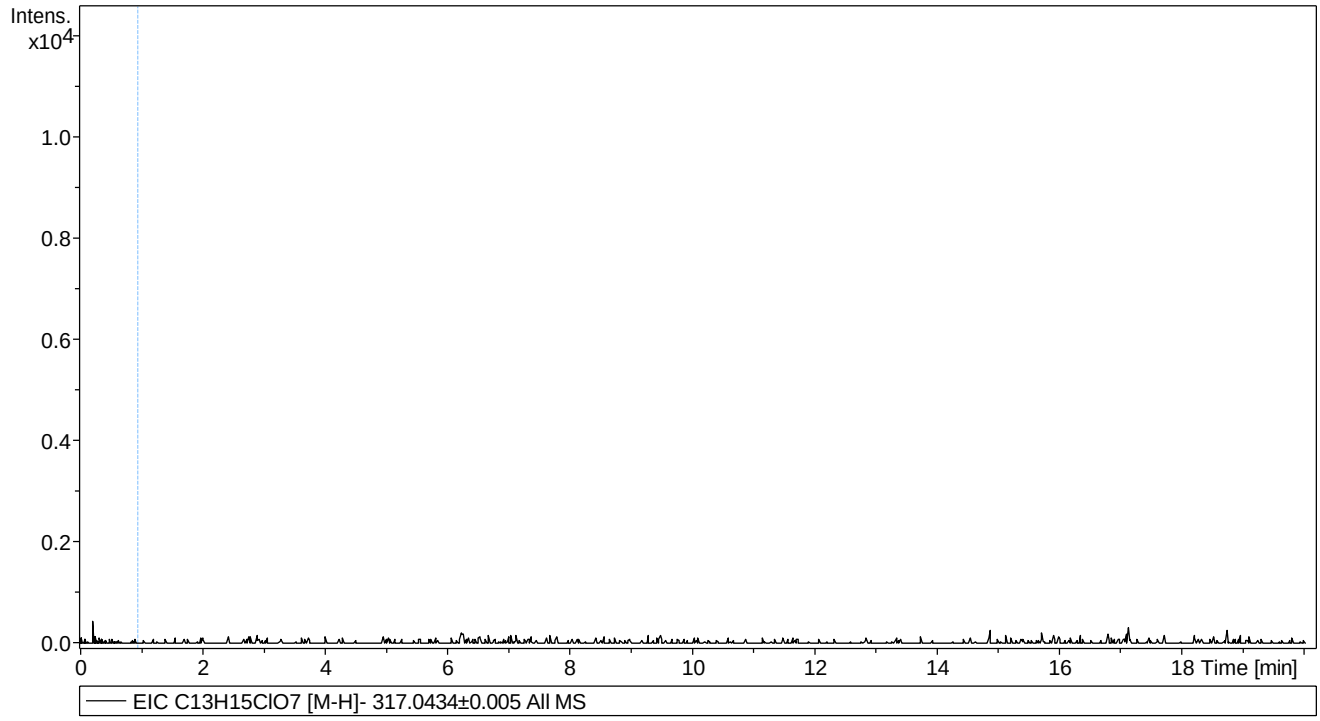
Operator BDAL@DE

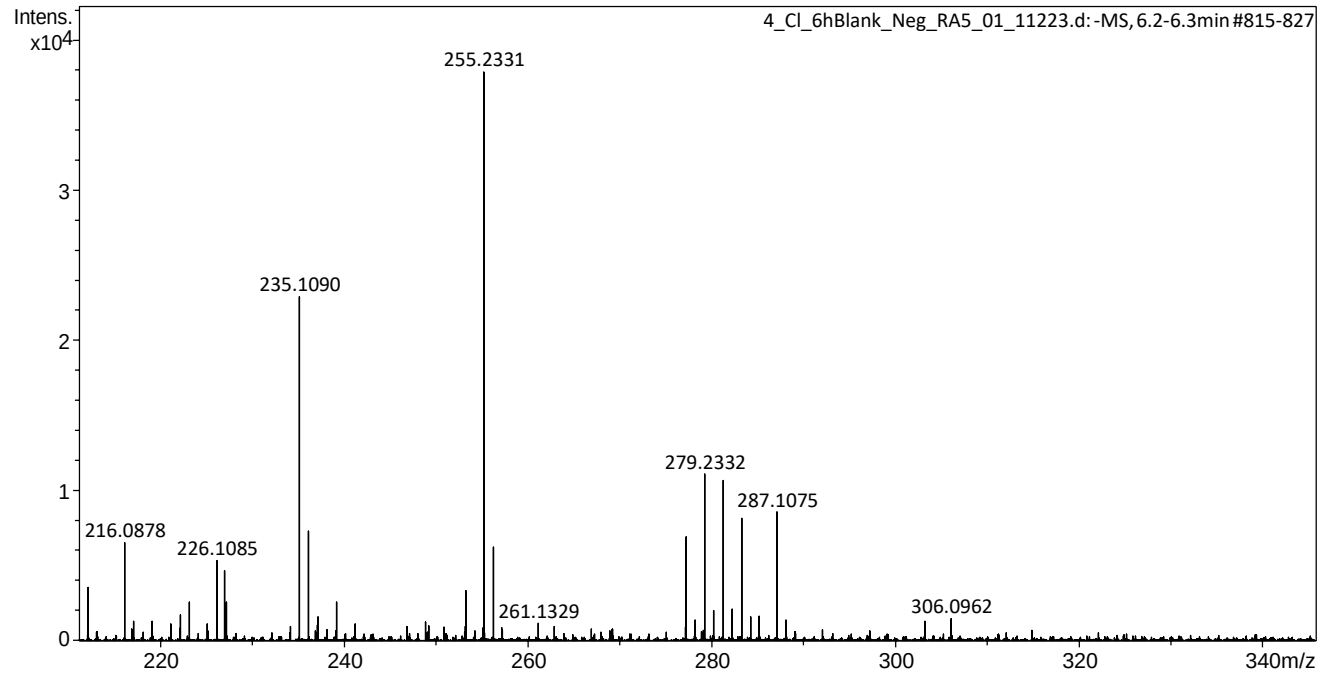
Sample Name 4_Cl_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

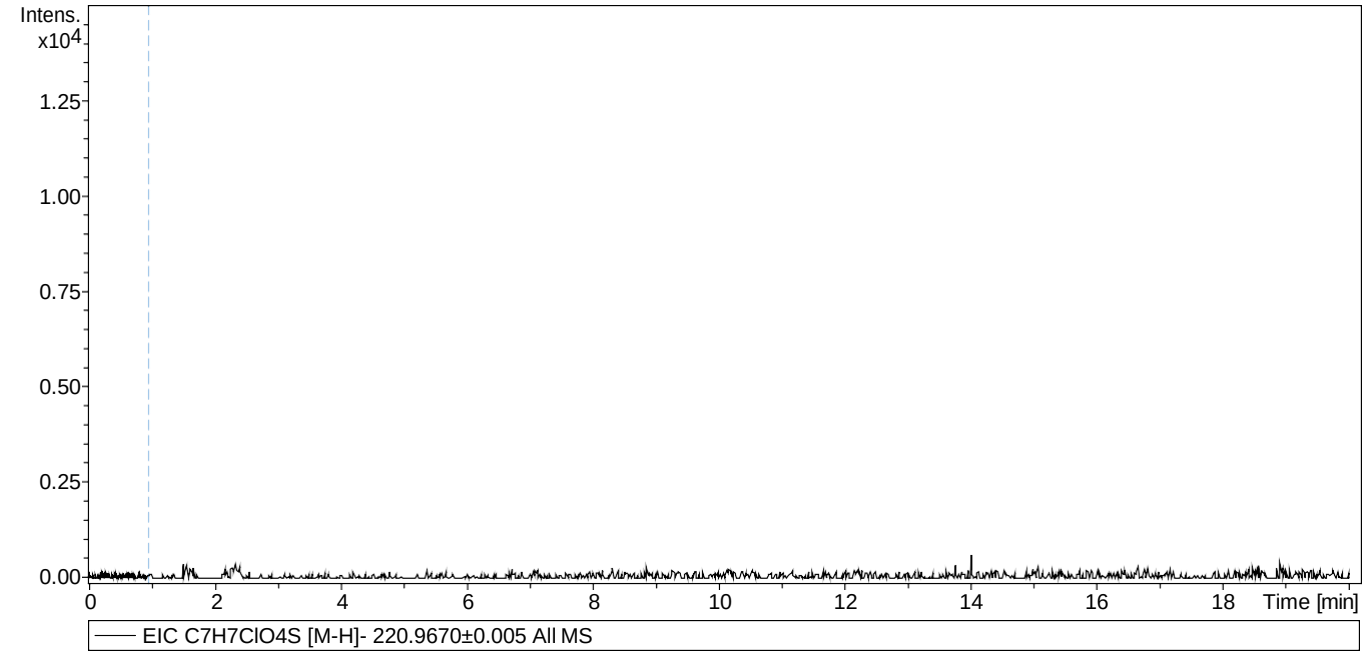
Acquisition Date 1/12/2016 5:23:29 PM

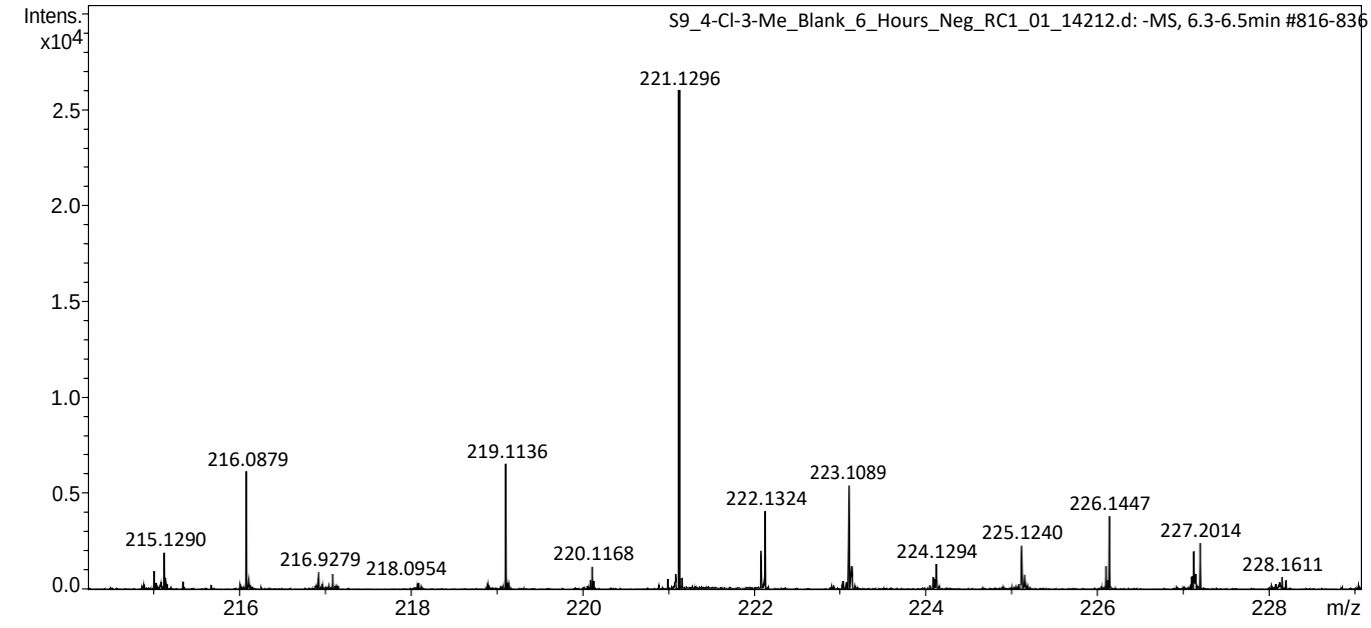
Sample Name S9_4-Cl-3-Me_Blank_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_Blank_6_Hours_Neg_RC1_01_14212.d

Display Report

Analysis Info

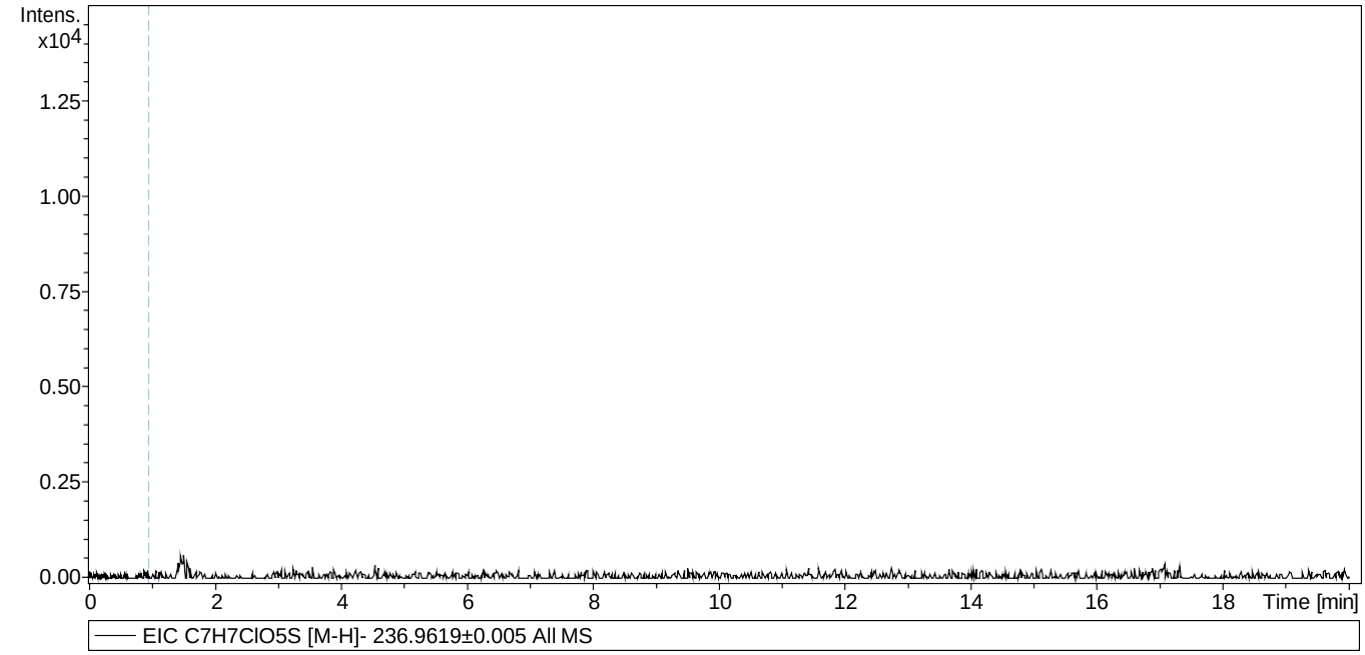
Acquisition Date 1/12/2016 5:23:29 PM

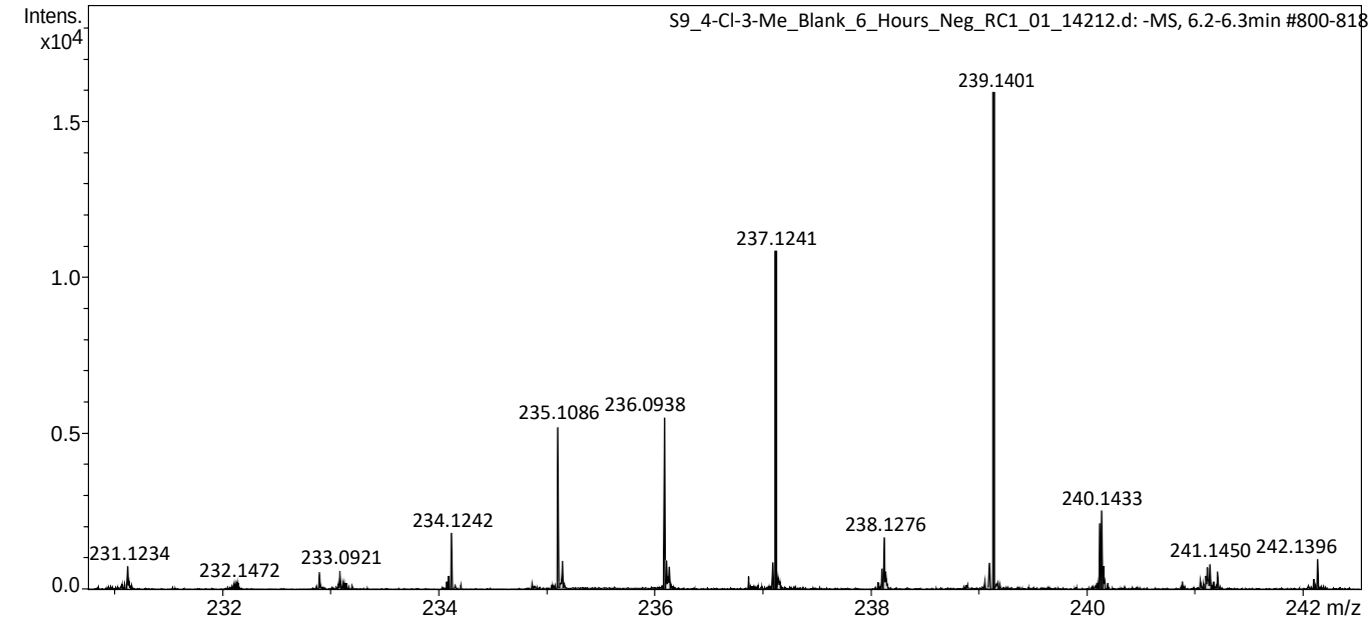
Sample Name S9_4-Cl-3-Me_Blank_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_Blank_6_Hours_Neg_RC1_01_14212.d

Display Report

Analysis Info

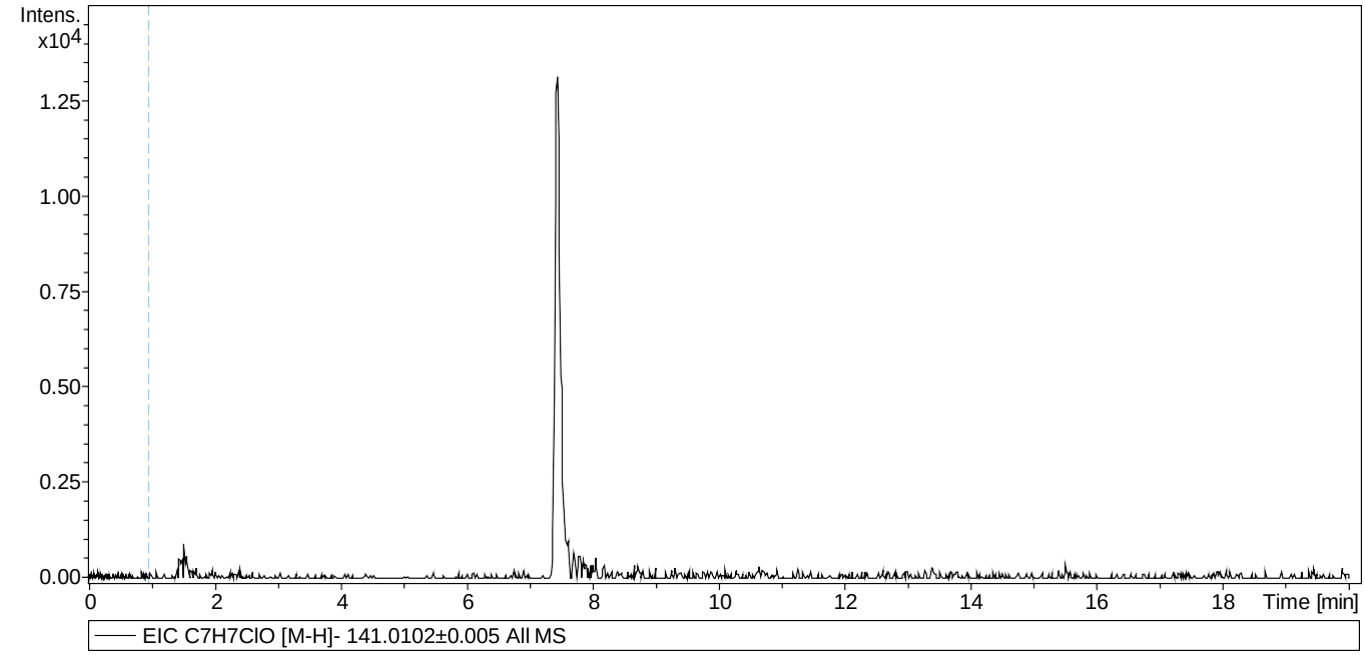
Acquisition Date 1/12/2016 5:23:29 PM

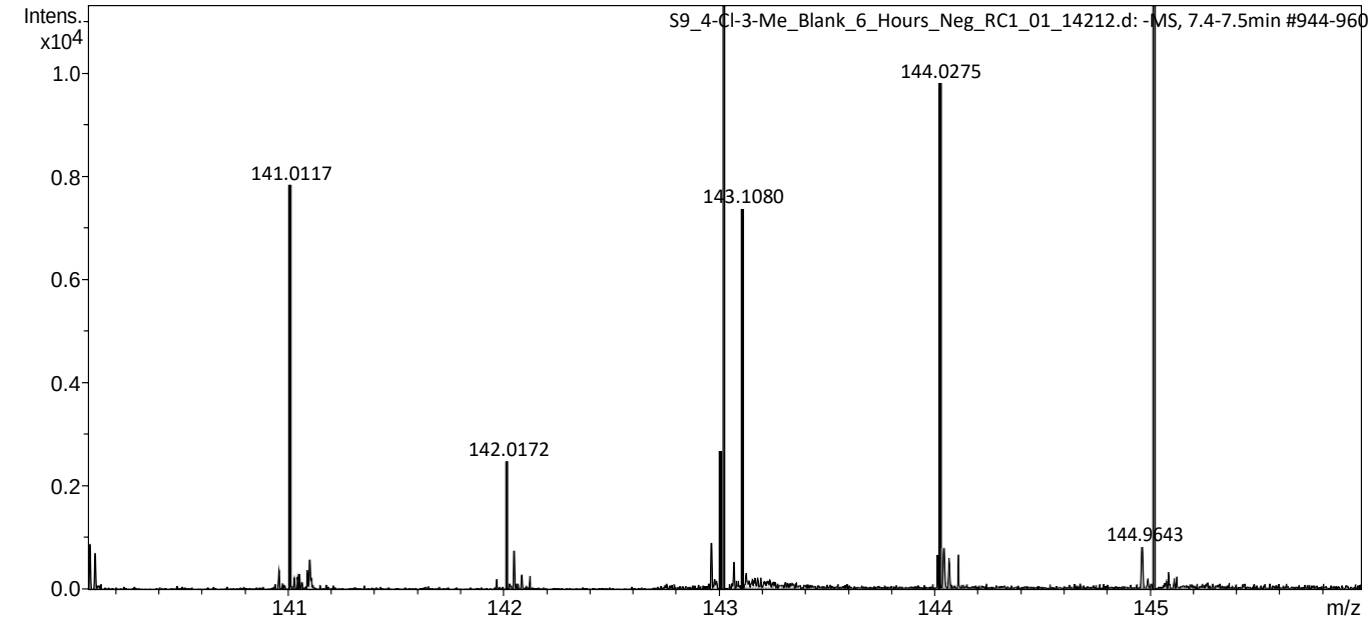
Sample Name S9_4-Cl-3-Me_Blank_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_Blank_6_Hours_Neg_RC1_01_14212.d

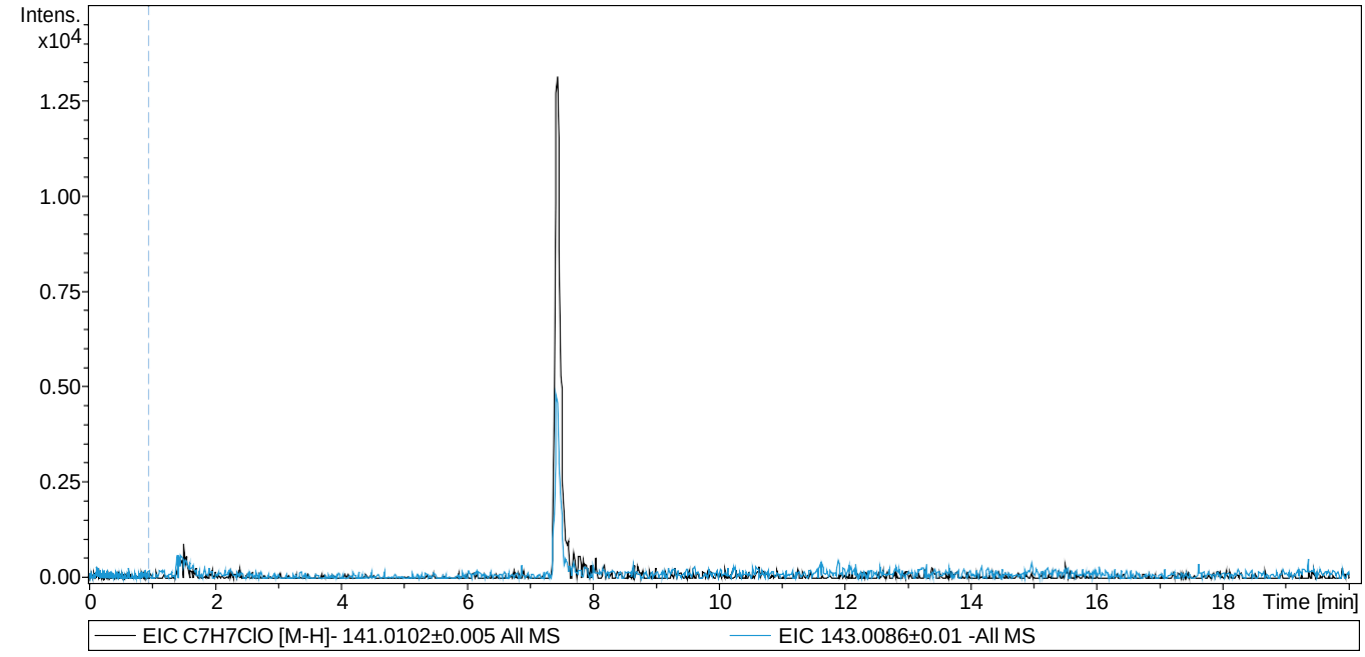
Display Report

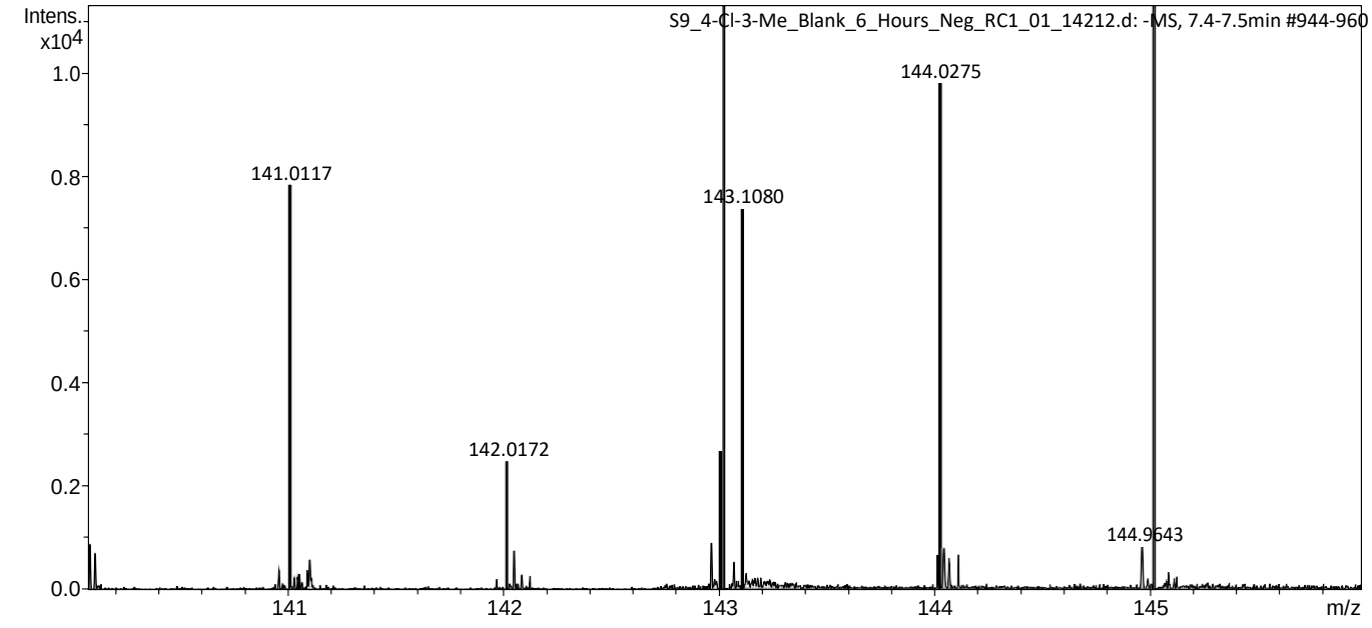
Analysis Info

Acquisition Date	1/12/2016 5:23:29 PM		
Sample Name	S9_4-Cl-3-Me_Blank_6_Hours_Neg	Operator	CCAF
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_4-Cl-3-Me_Blank_6_Hours_Neg_RC1_01_14212.d

Display Report

Analysis Info

Acquisition Date 11/23/2016 9:03:25 PM

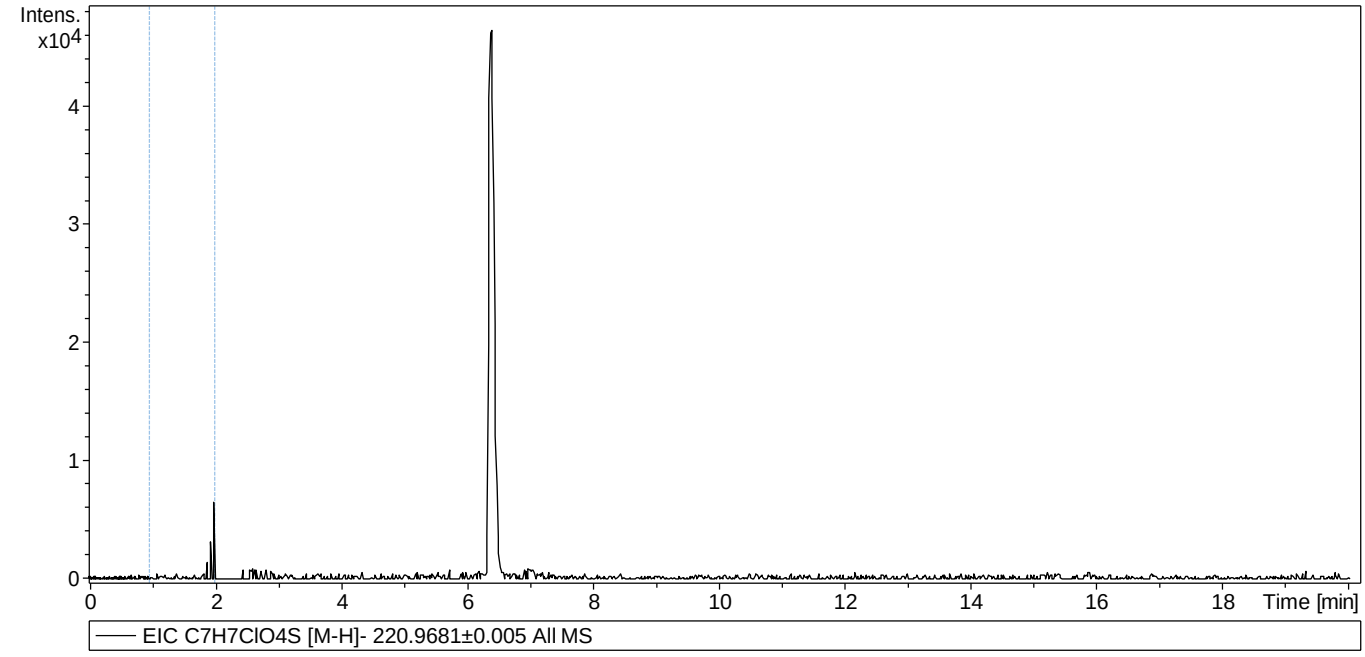
Sample Name Urine_141_A neg

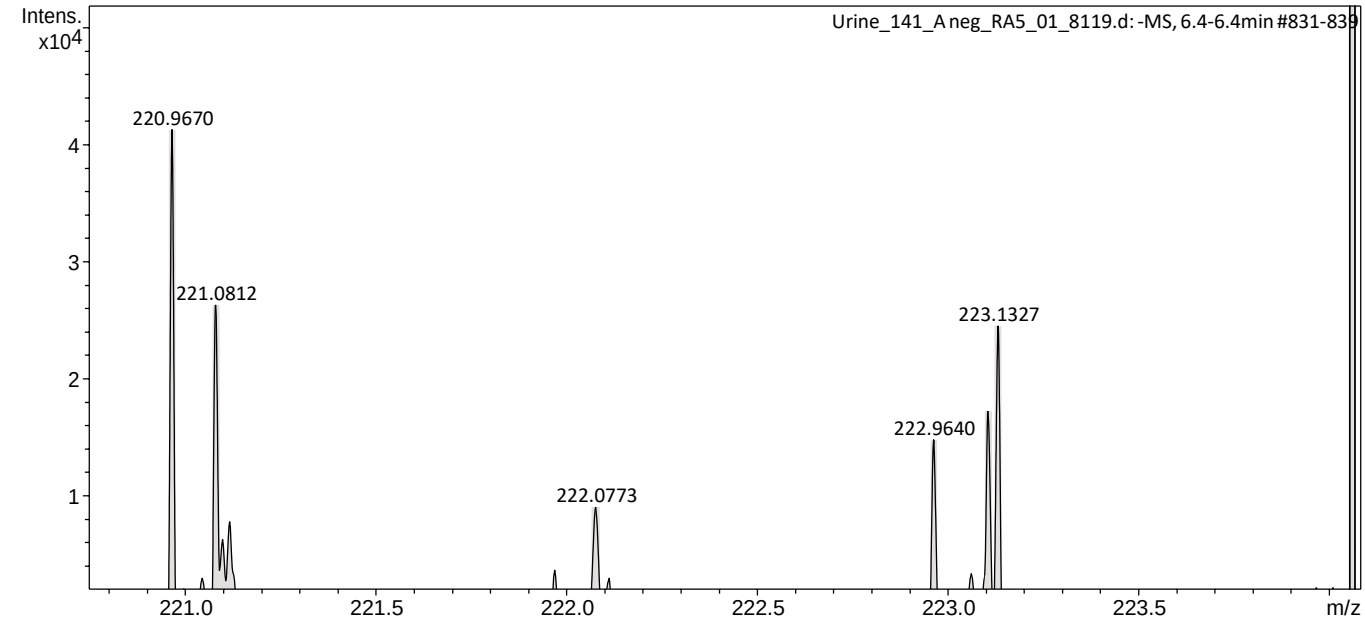
Operator BDAL@DE

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_A neg_RA5_01_8119.d

Display Report

Analysis Info

Acquisition Date 11/23/2016 9:03:25 PM

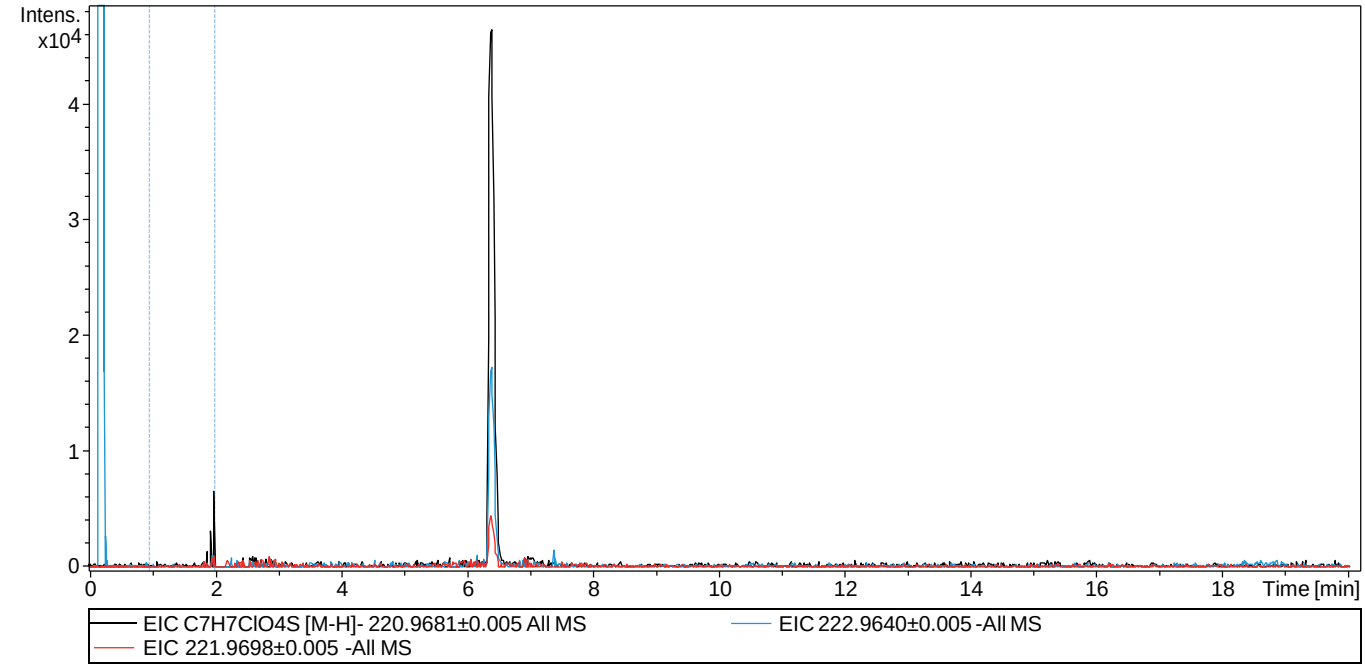
Sample Name Urine_141_A neg

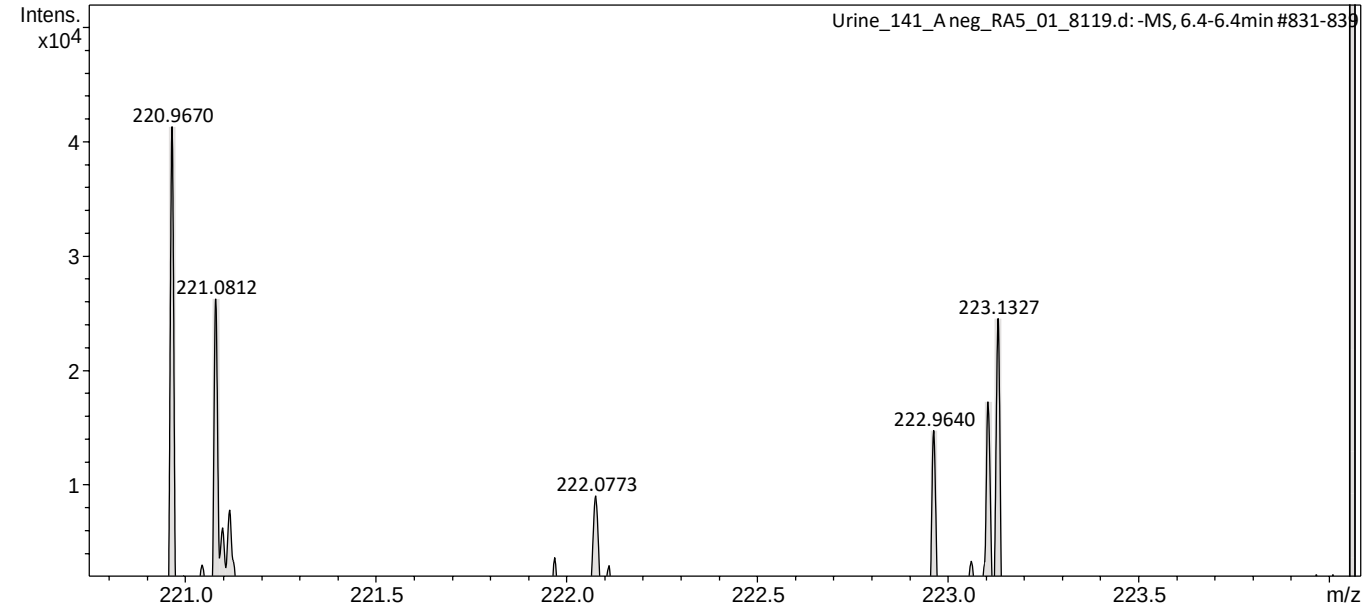
Operator BDAL@DE

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_A neg_RA5_01_8119.d

Display Report

Analysis Info

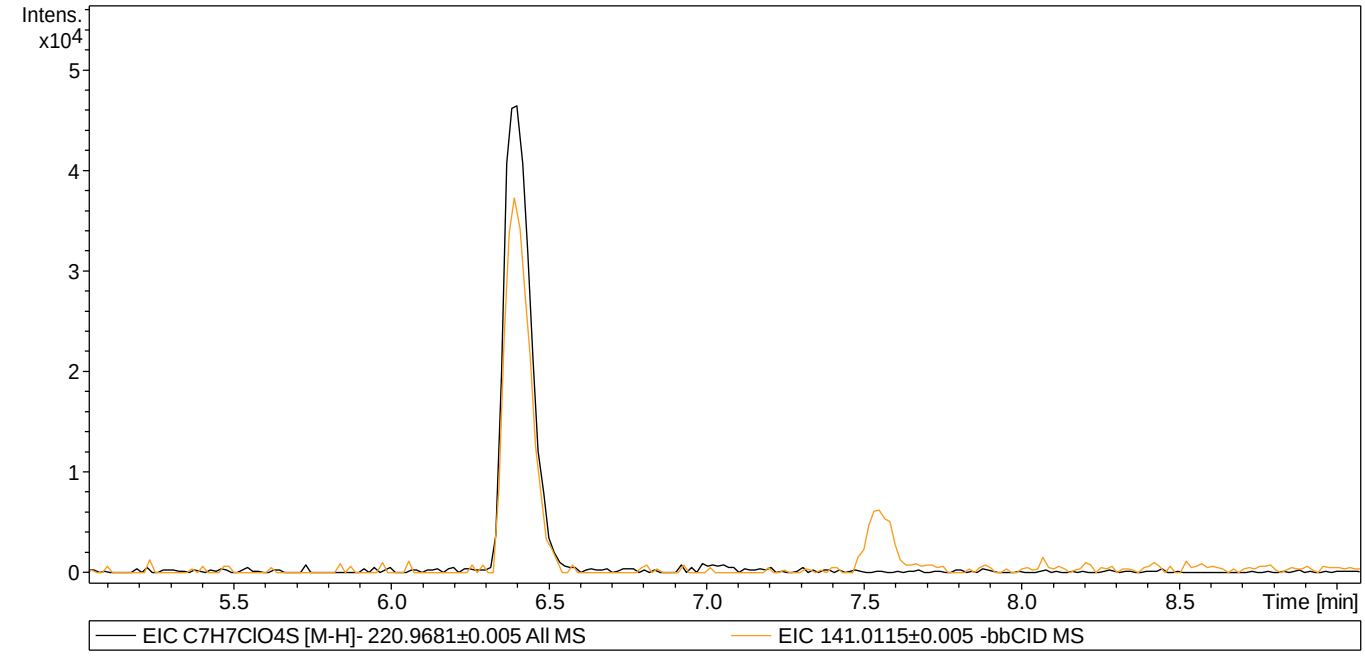
Acquisition Date 11/23/2016 9:03:25 PM

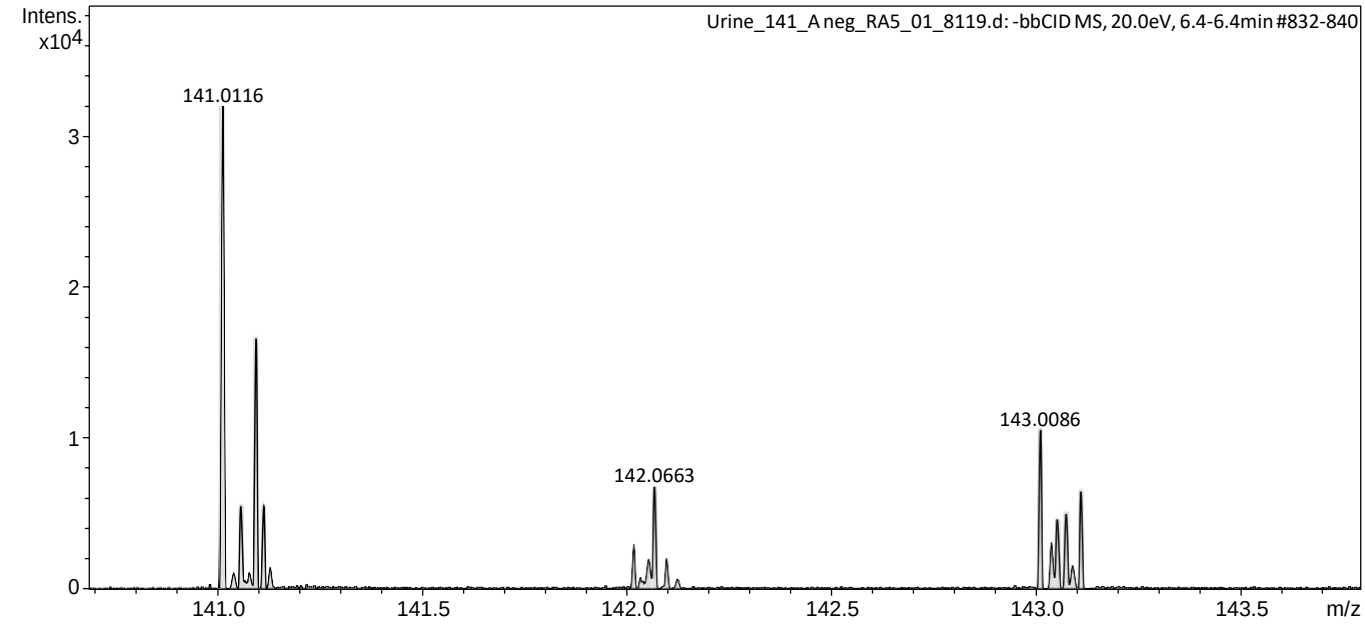
Sample Name Urine_141_A neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_A neg_RA5_01_8119.d

Display Report

Analysis Info

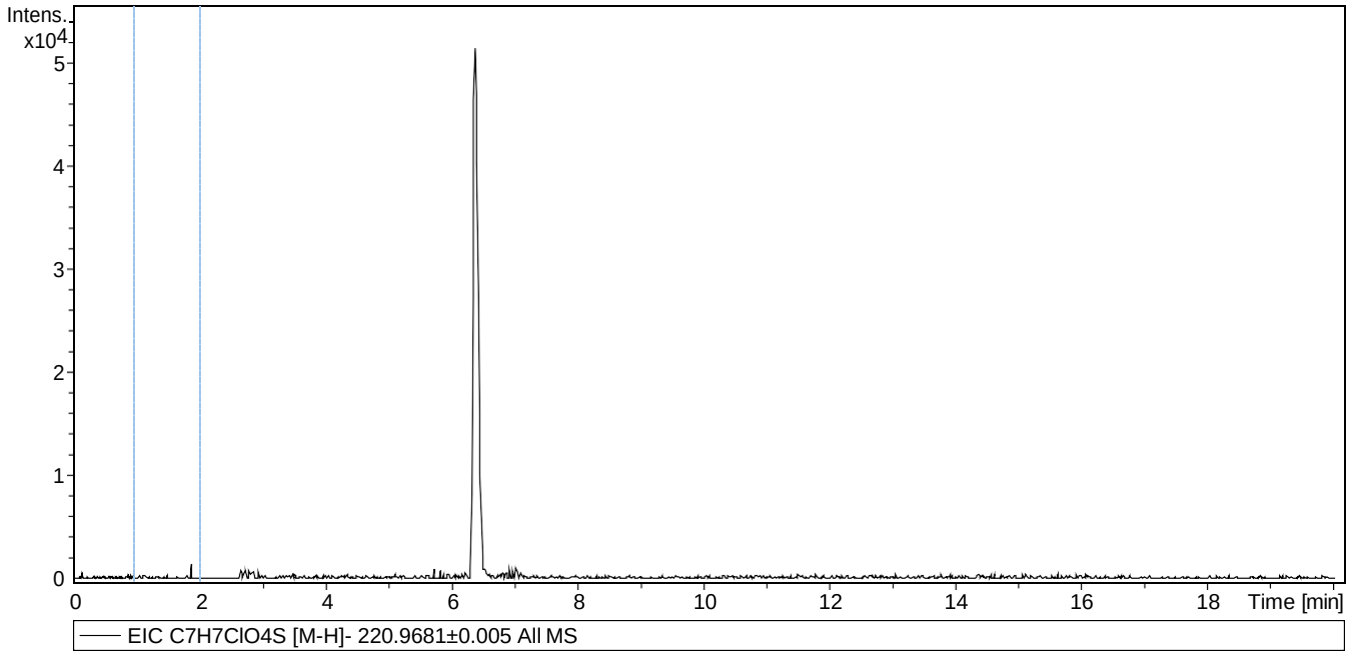
Acquisition Date 11/23/2016 9:45:56 PM

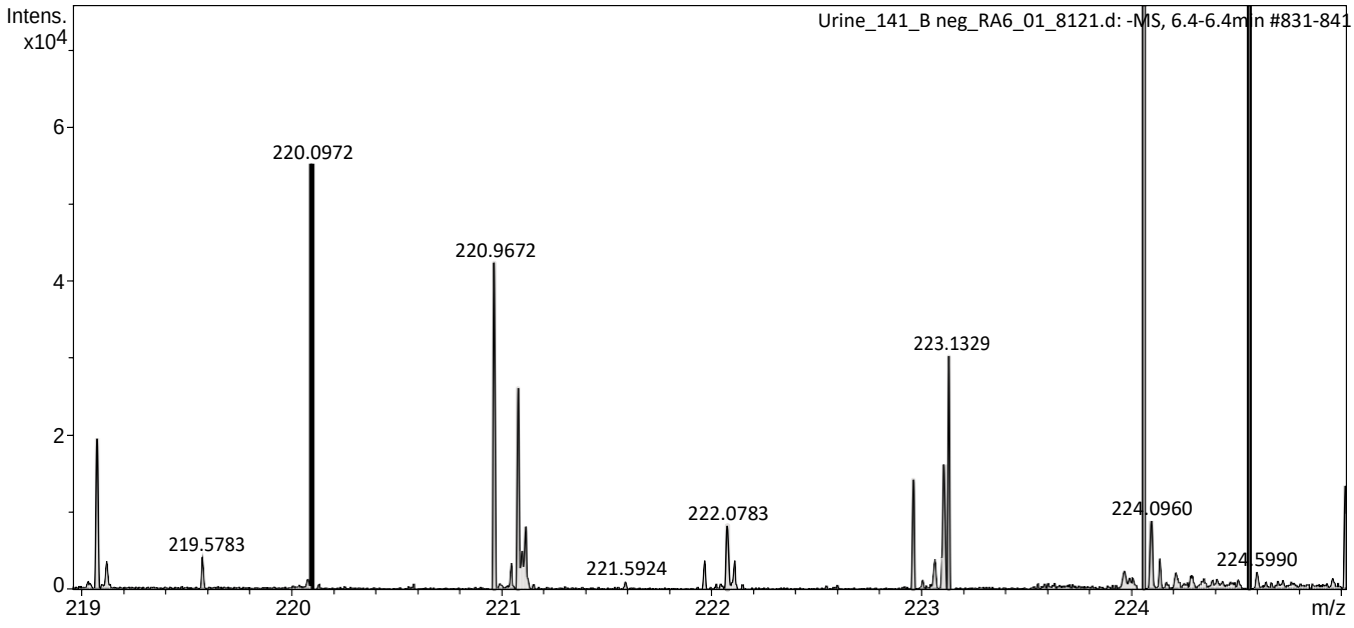
Sample Name Urine_141_B neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_B neg_RA6_01_8121.d

Display Report

Analysis Info

Acquisition Date 11/23/2016 9:45:56 PM

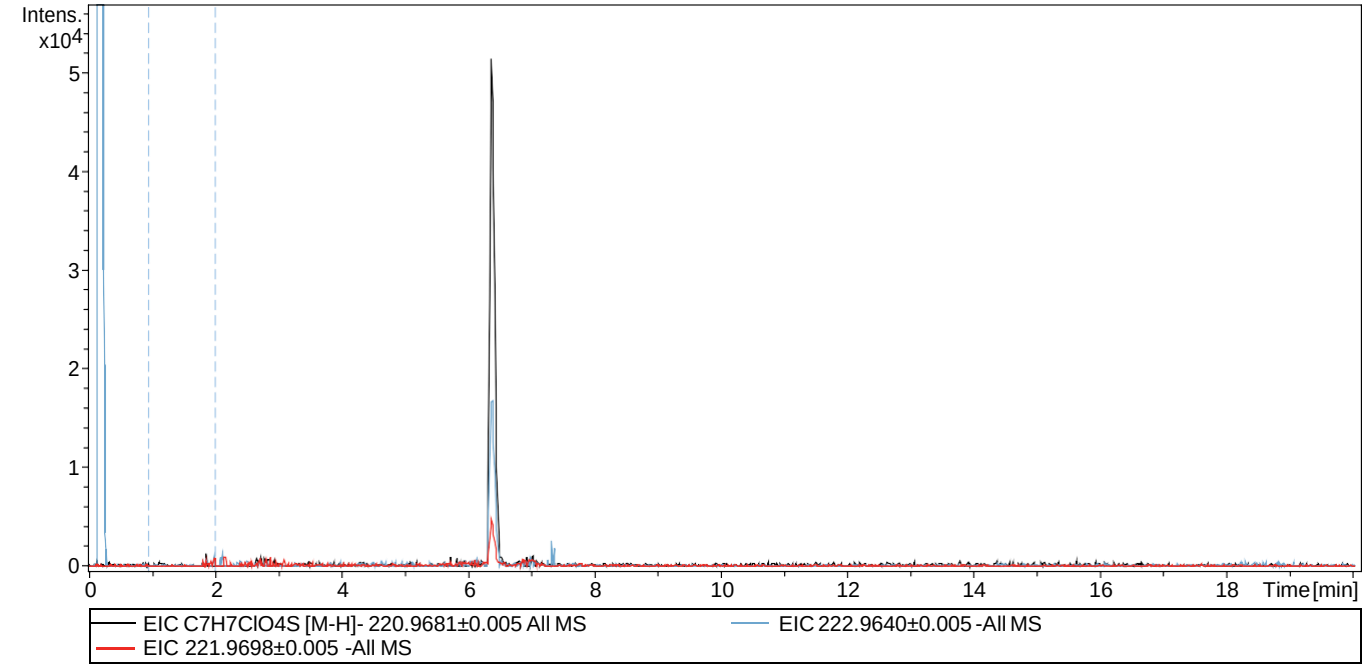
Sample Name Urine_141_B neg

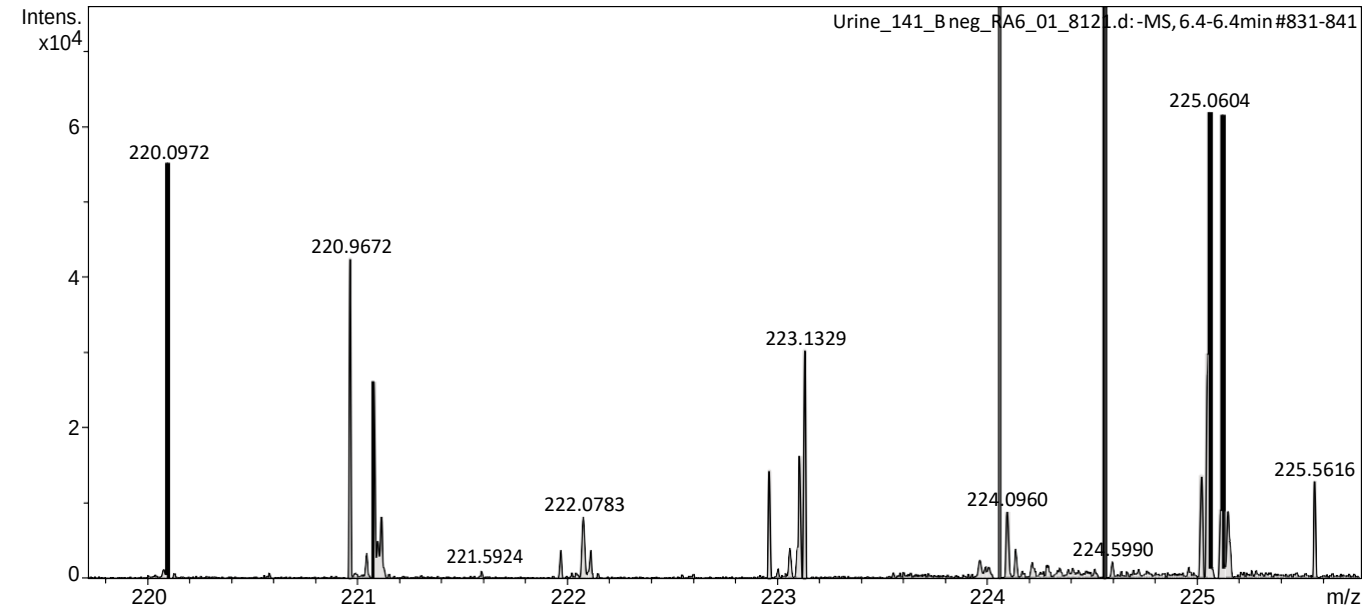
Operator BDAL@DE

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_B neg_RA6_01_8121.d

Display Report

Analysis Info

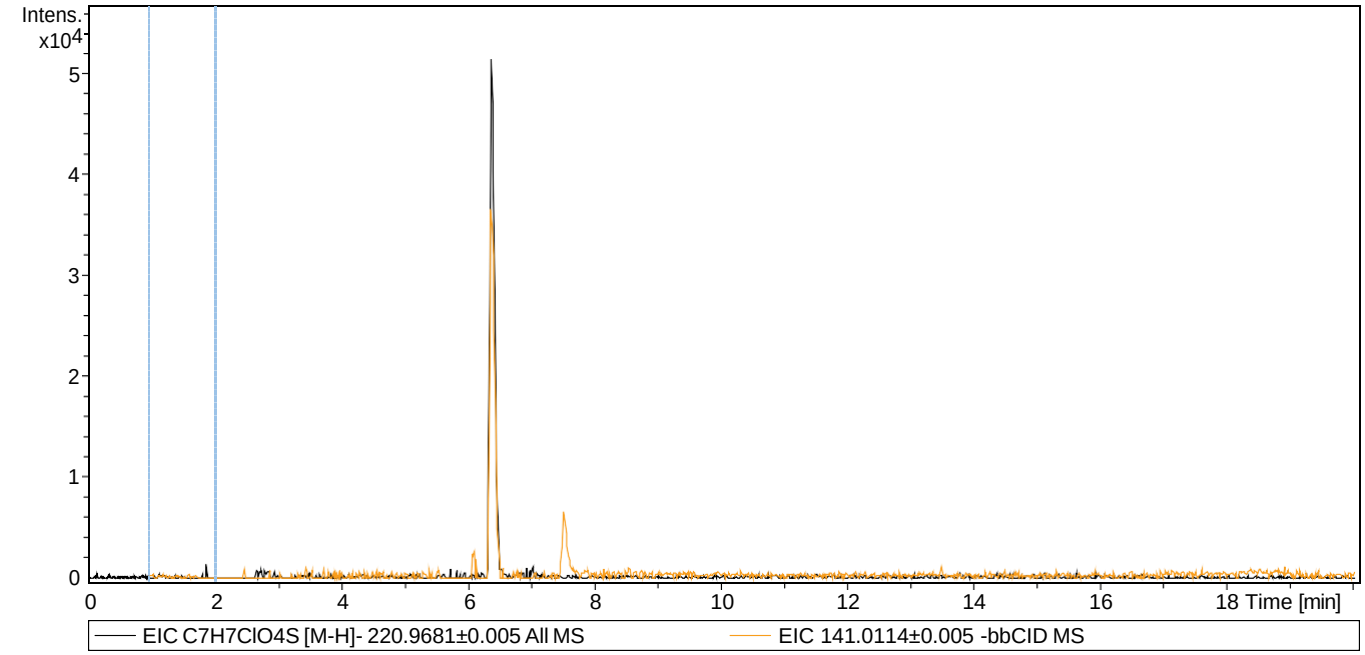
Acquisition Date 11/23/2016 9:45:56 PM

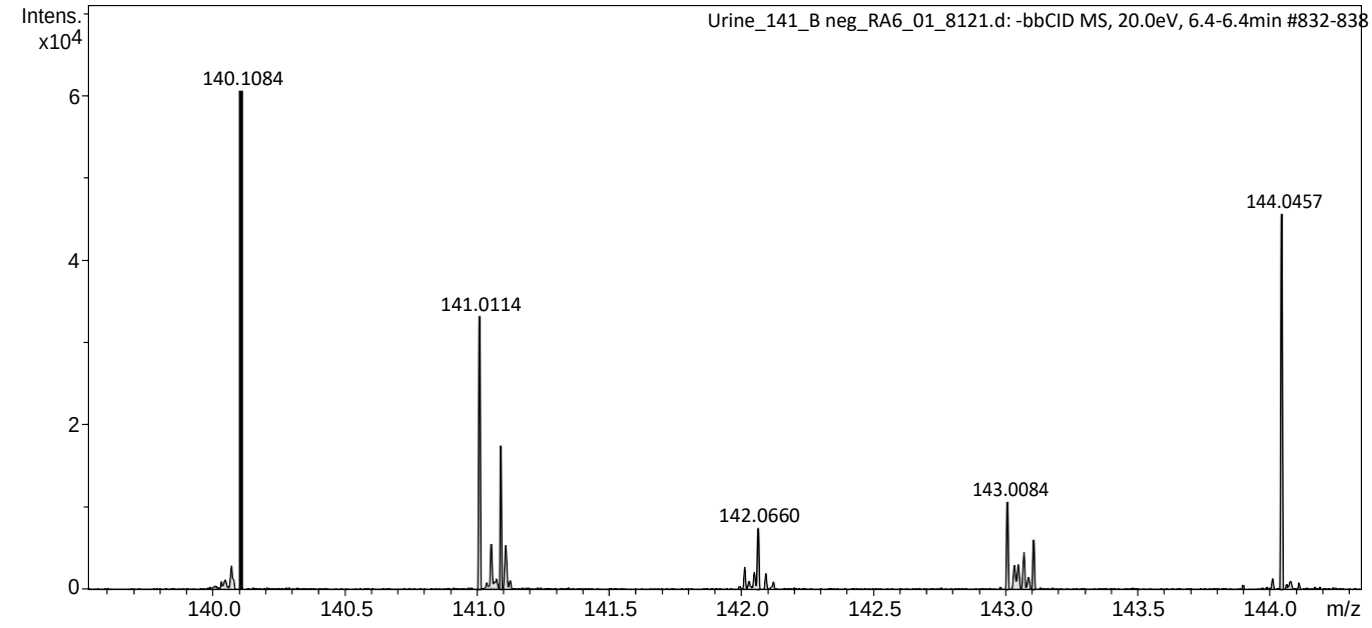
Sample Name Urine_141_B neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





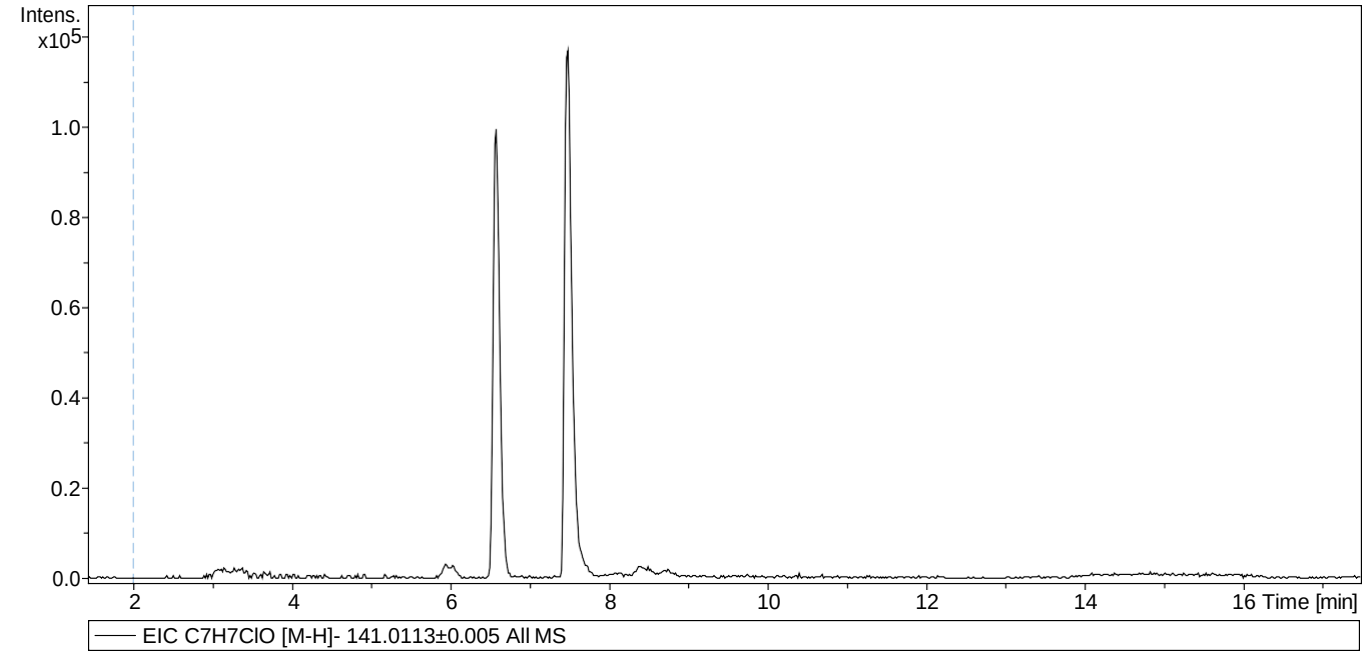
Display Report

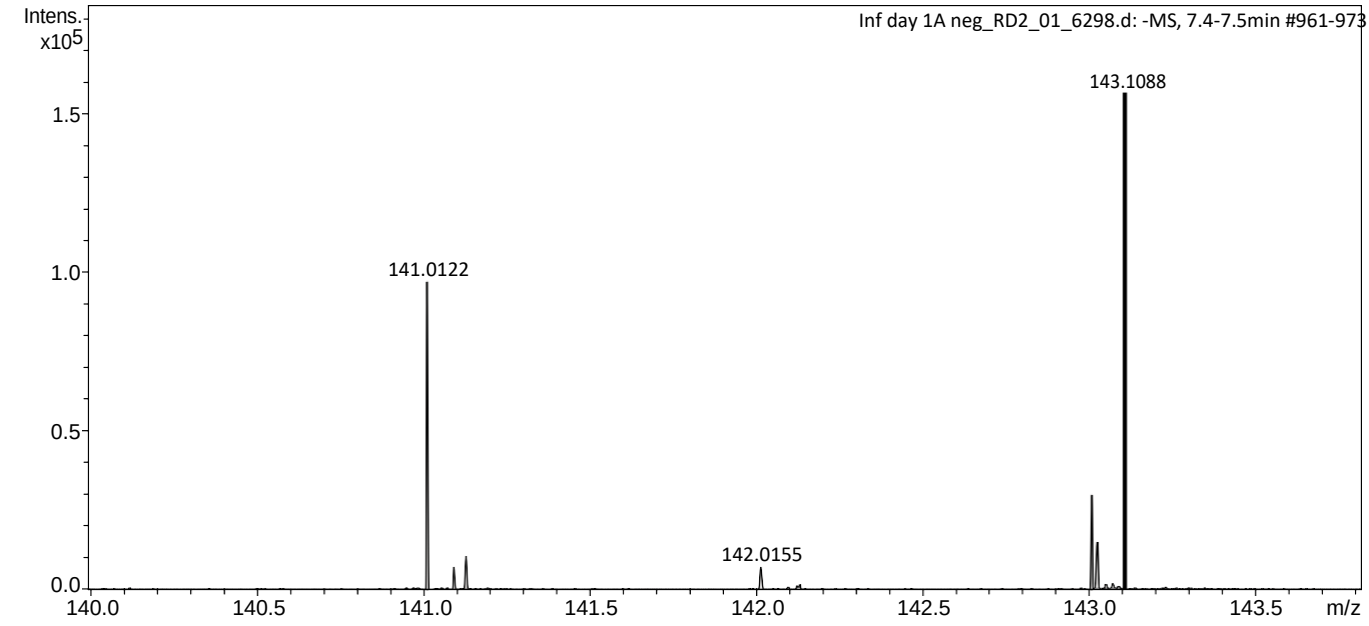
Analysis Info

Acquisition Date	10/19/2016 8:26:24 PM		
Sample Name	Inf day 1A neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

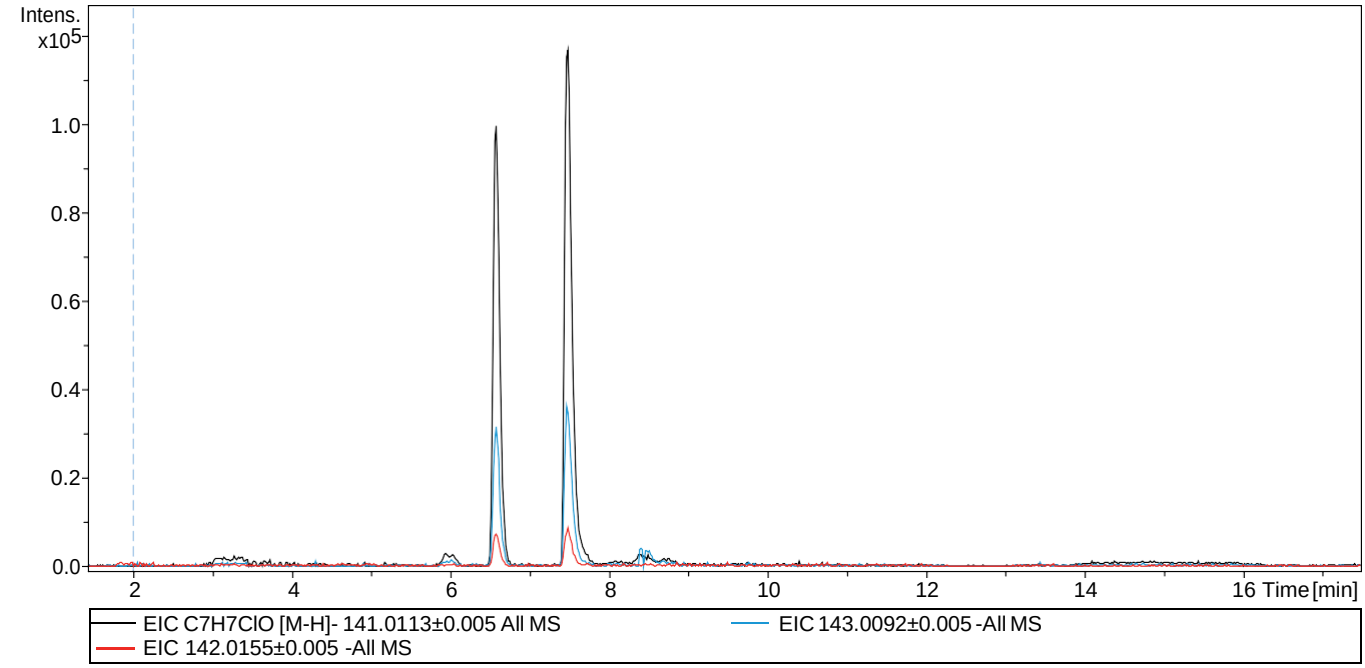
Display Report

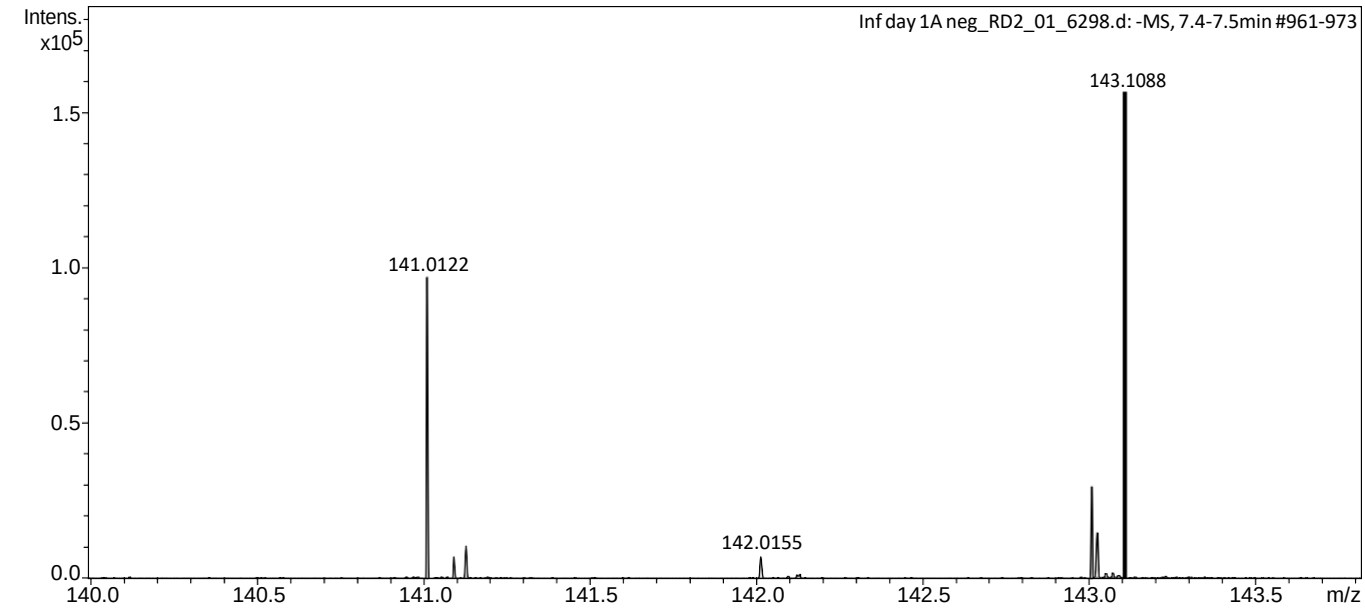
Analysis Info

Acquisition Date	10/19/2016 8:26:24 PM		
Sample Name	Inf day 1A neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

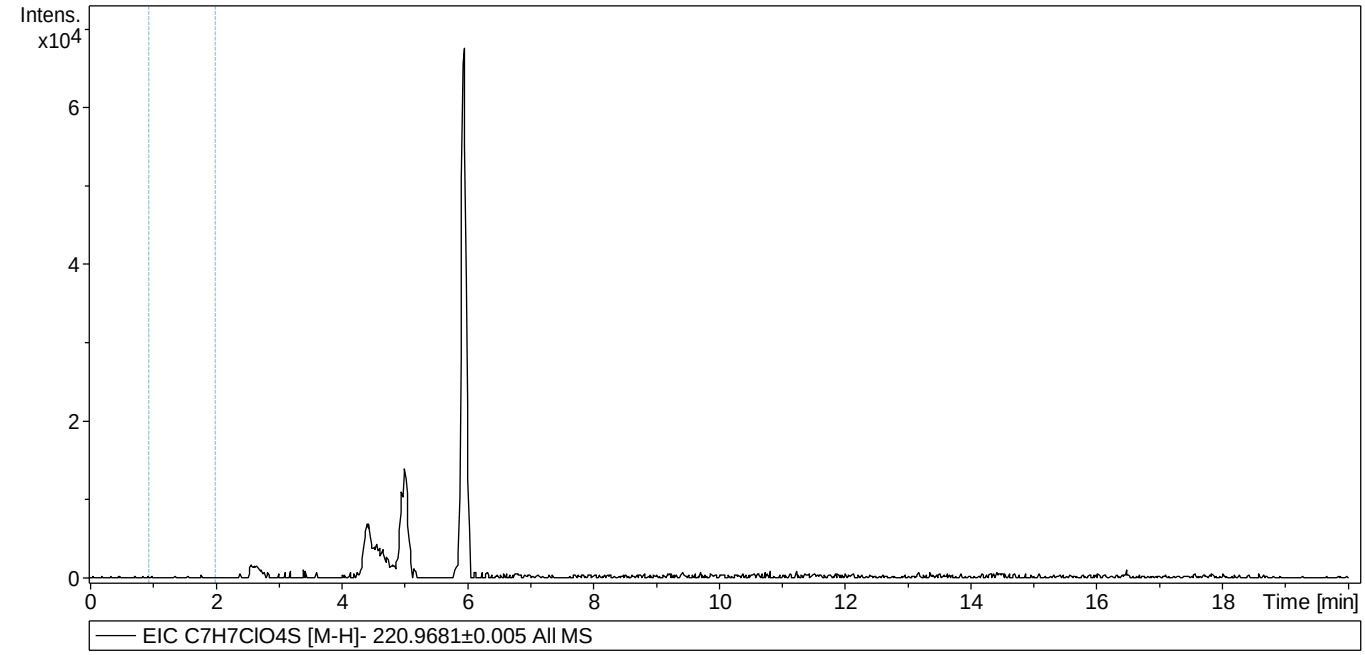
Display Report

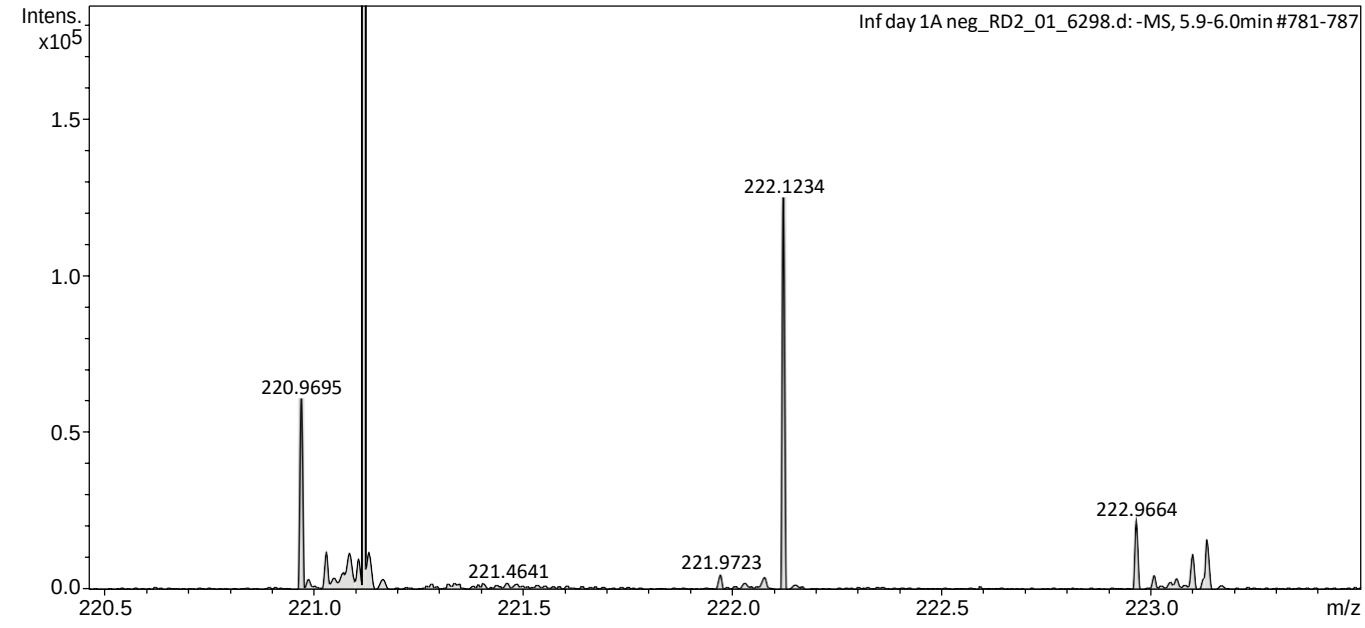
Analysis Info

Acquisition Date	10/19/2016 8:26:24 PM		
Sample Name	Inf day 1A neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

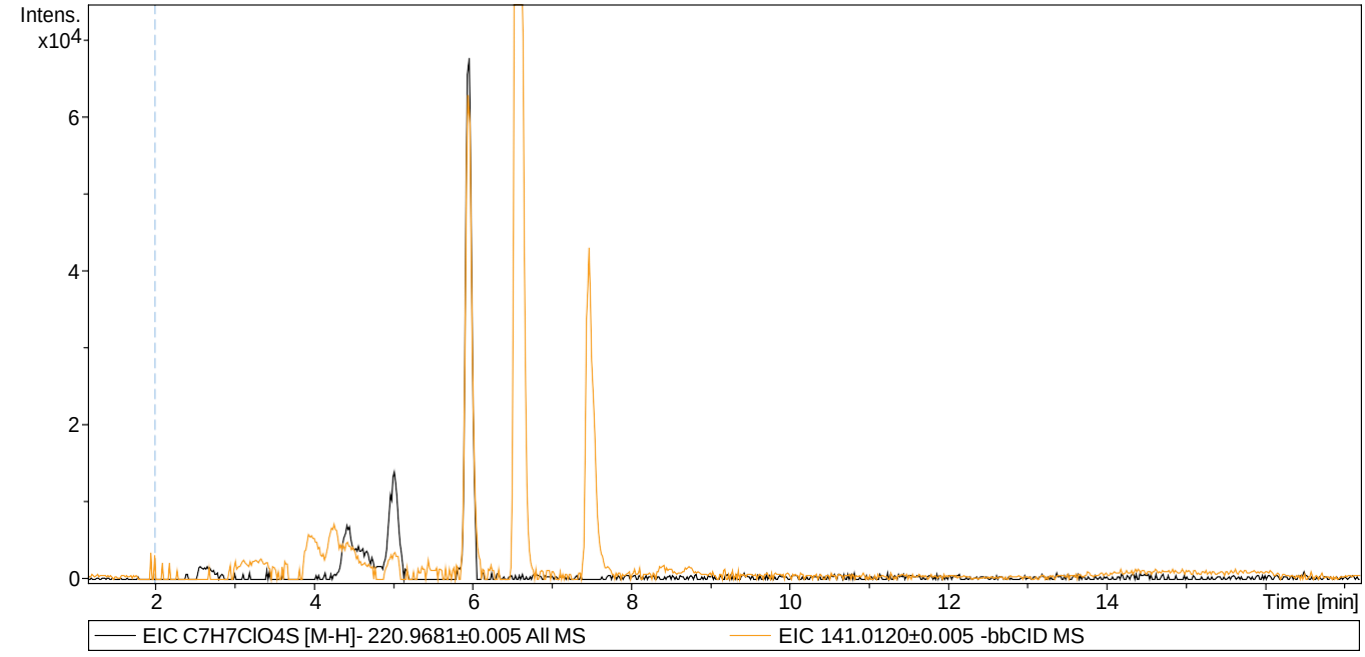
Display Report

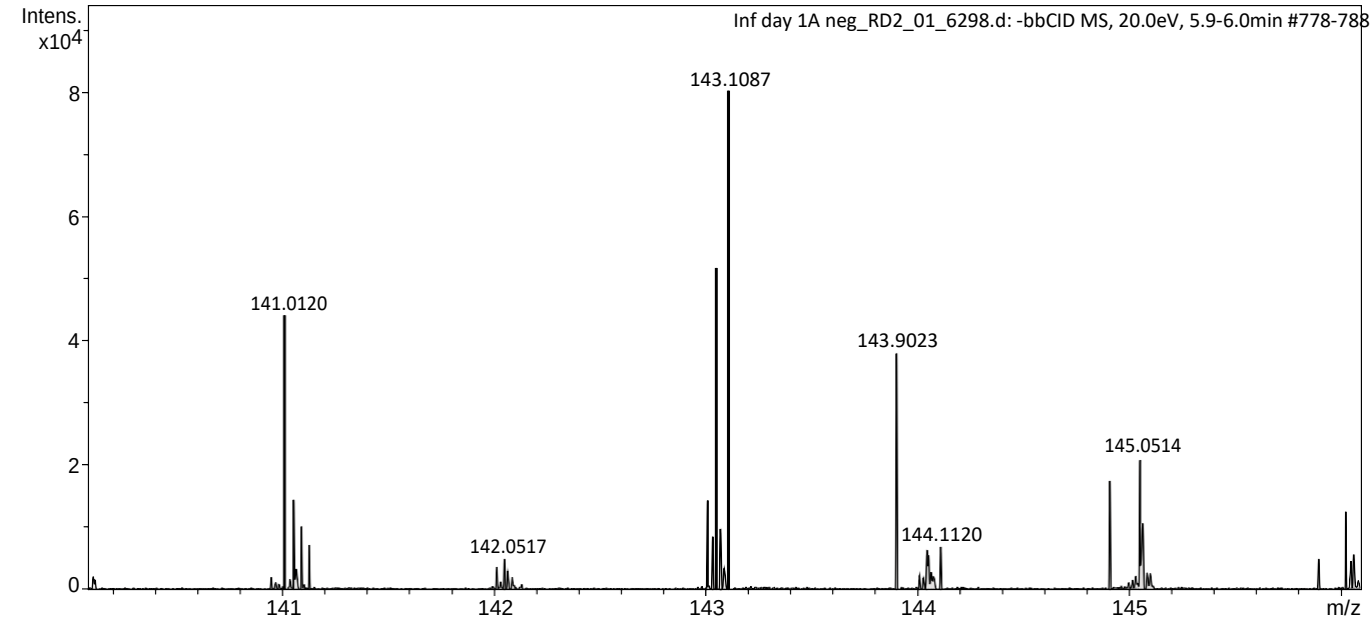
Analysis Info

Acquisition Date	10/19/2016 8:26:24 PM		
Sample Name	Inf day 1A neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

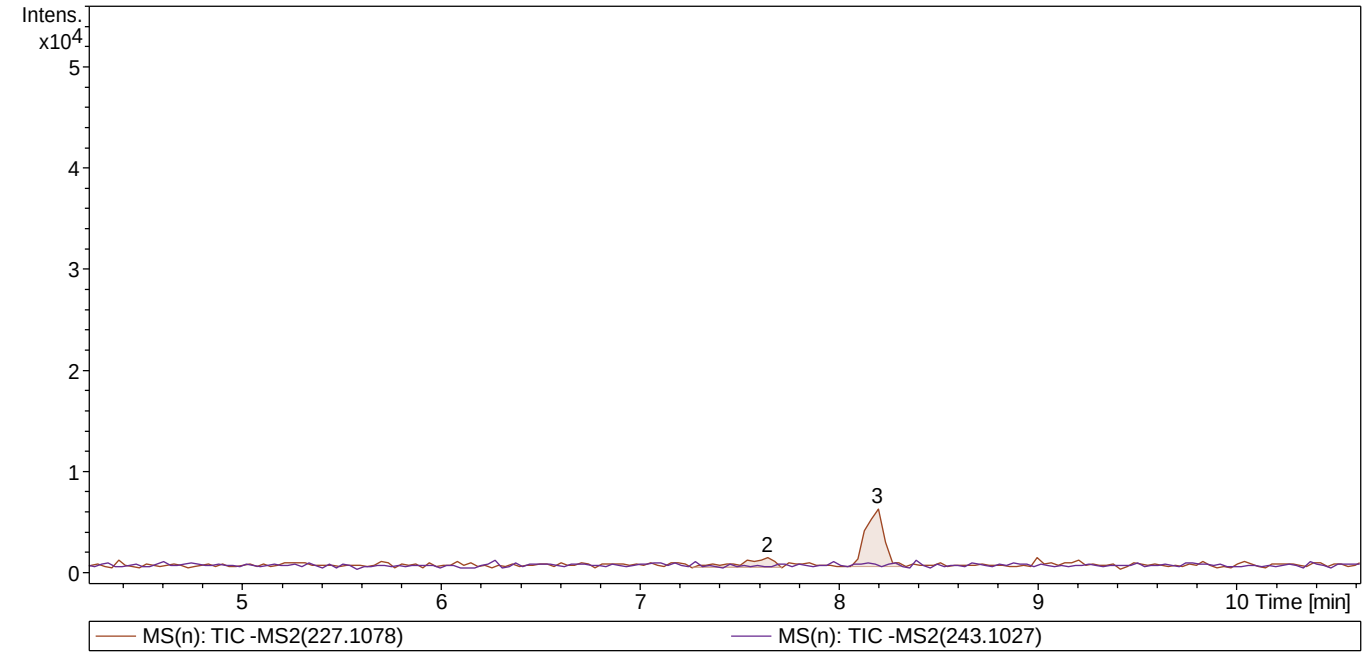
Display Report

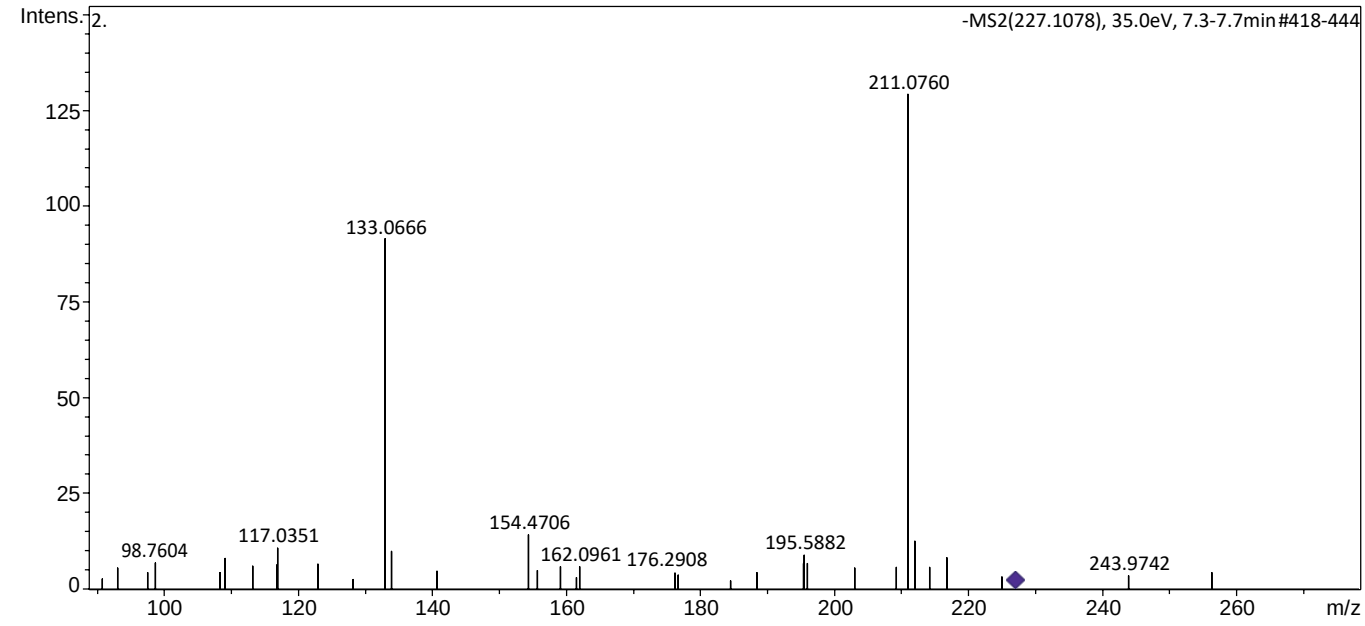
Analysis Info

Acquisition Date	7/20/2016 6:17:37 PM		
Sample Name	MRM_BPAandBPAox_Met1_testSTD_2	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	4.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	12.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





MRM_BPAandBPAox_Met1_testSTD_2_RA2_01_4598.d

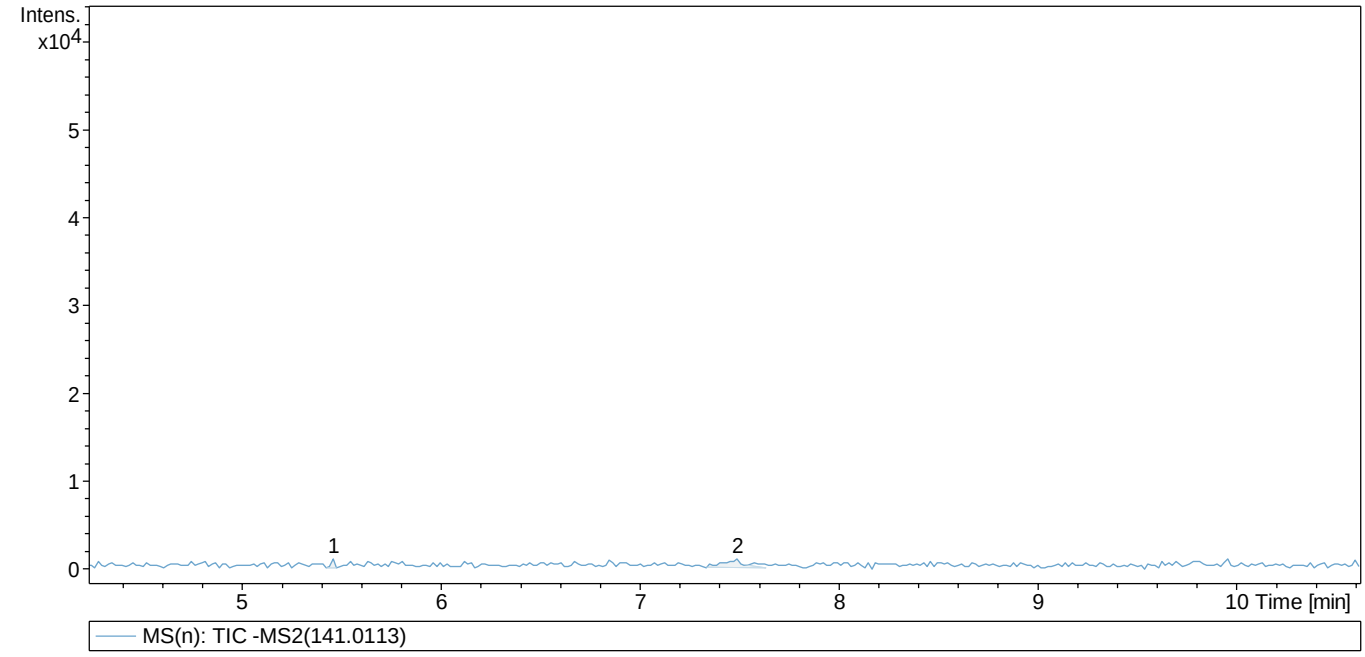
Display Report

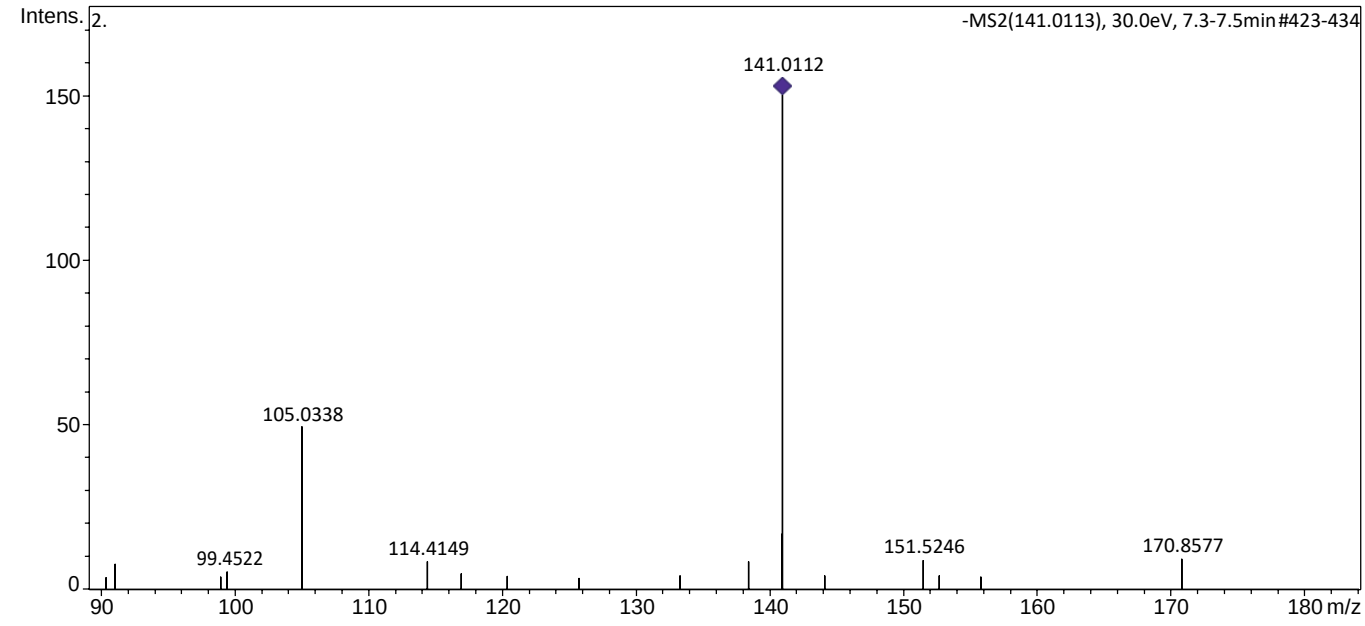
Analysis Info

Acquisition Date	7/21/2016 5:00:16 PM		
Sample Name	MRM_4Cl3MPox_Met2_STD_5	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	4.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	12.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





MRM_4Cl3MPox_Met2_STD_5_RA2_01_4618.d

Appendix B: List of reports

Report 1 XICs and mass spectra of BPA metabolites identified following in vitro experiments
Report 2 XICs and mass spectra of BP1 metabolites identified following in vitro experiments
Report 3 XICs and mass spectra of BP2 metabolites identified following in vitro experiments
Report 4 XICs and mass spectra of 4,4-DHBP metabolites identified following in vitro experiments
Report 5 XICs and mass spectra of 4-BenzPh metabolites identified following in vitro experiments
Report 6 XICs and mass spectra of HO metabolites identified following in vitro experiments
Report 7 XICs and mass spectra of OC metabolites identified following in vitro experiments
Report 8 XICs and mass spectra of 3BC metabolites identified following in vitro experiments
Report 9 XICs and mass spectra of compounds identified in urine
Report 10 XICs and mass spectra of compounds identified in wastewater

Display Report

Analysis Info

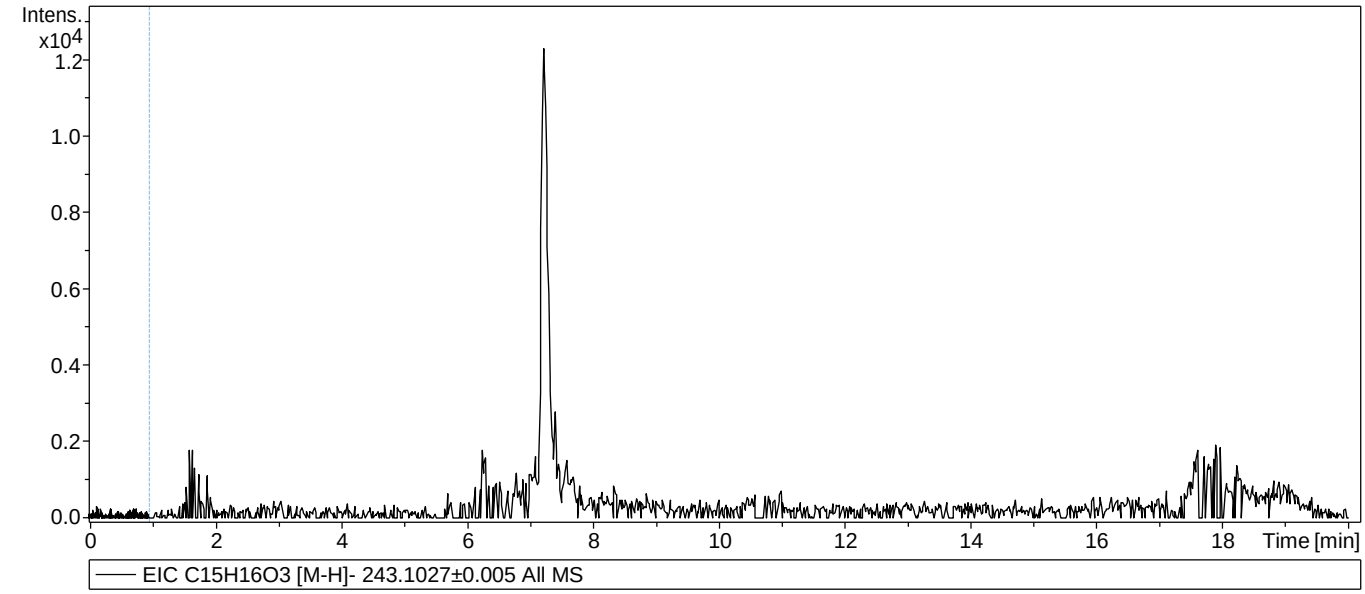
Acquisition Date 3/24/2016 11:38:56 PM

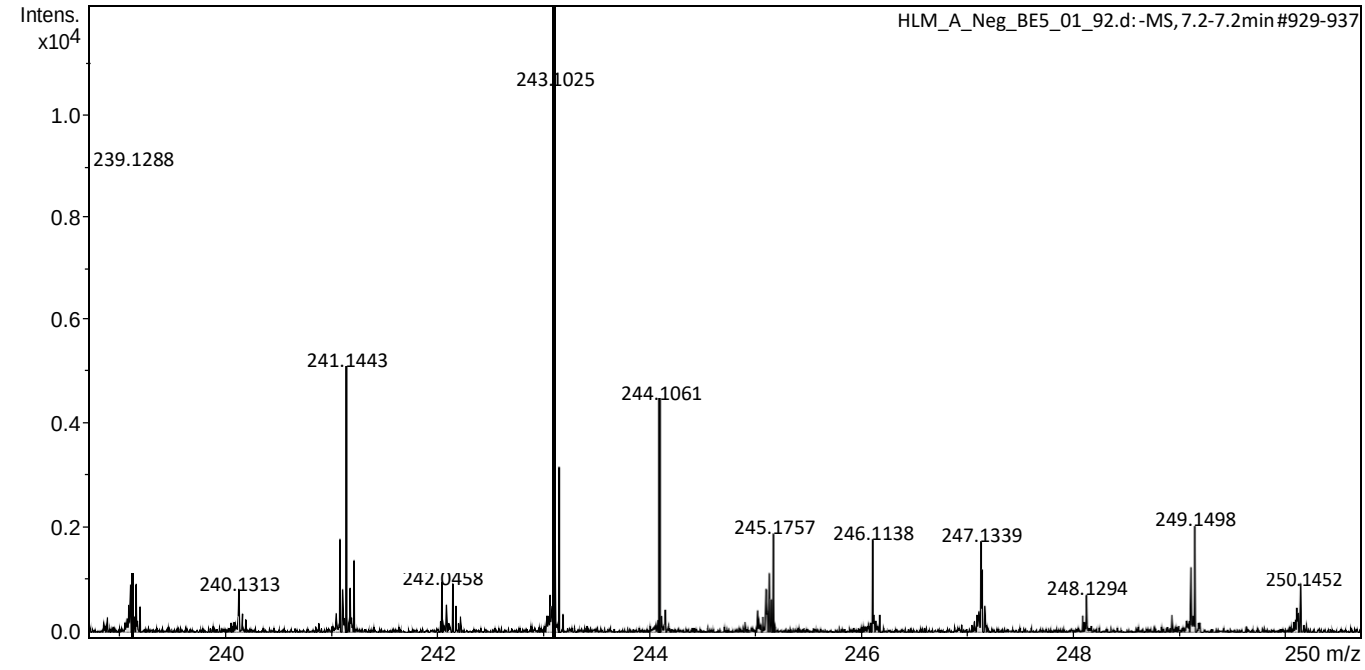
Sample Name HLM_A_Neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





HLM_A_Neg_BE5_01_92.d

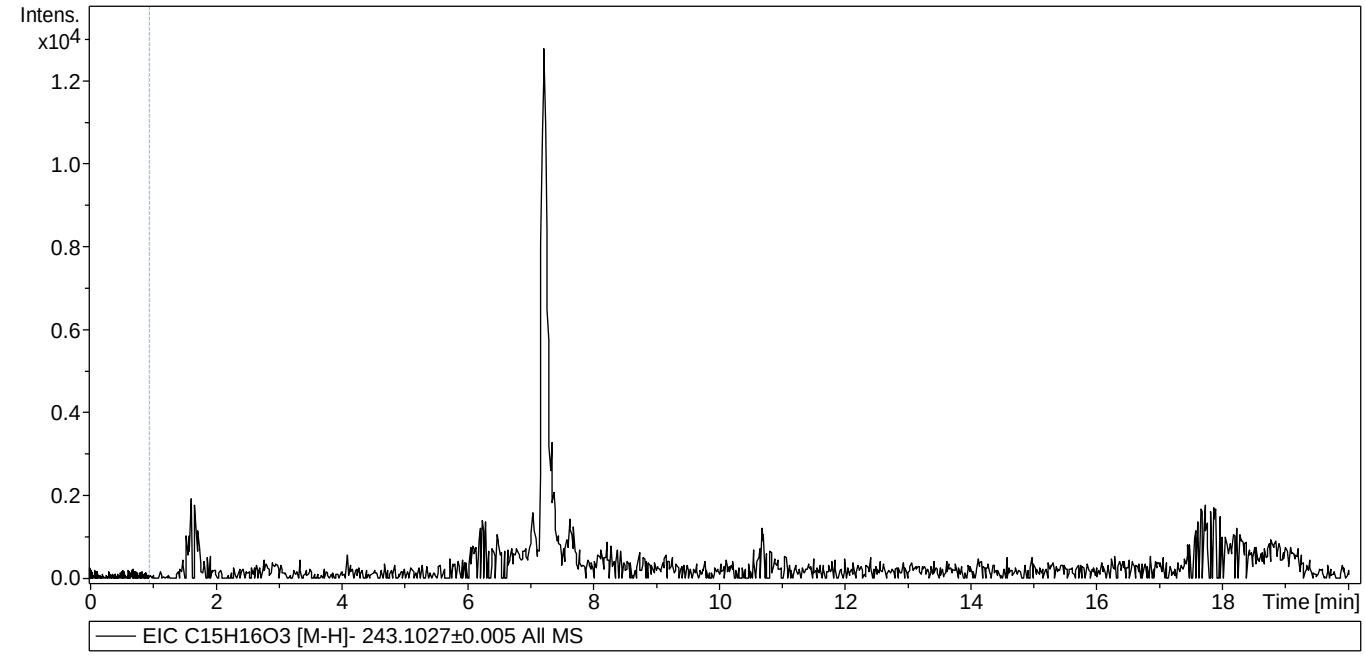
Display Report

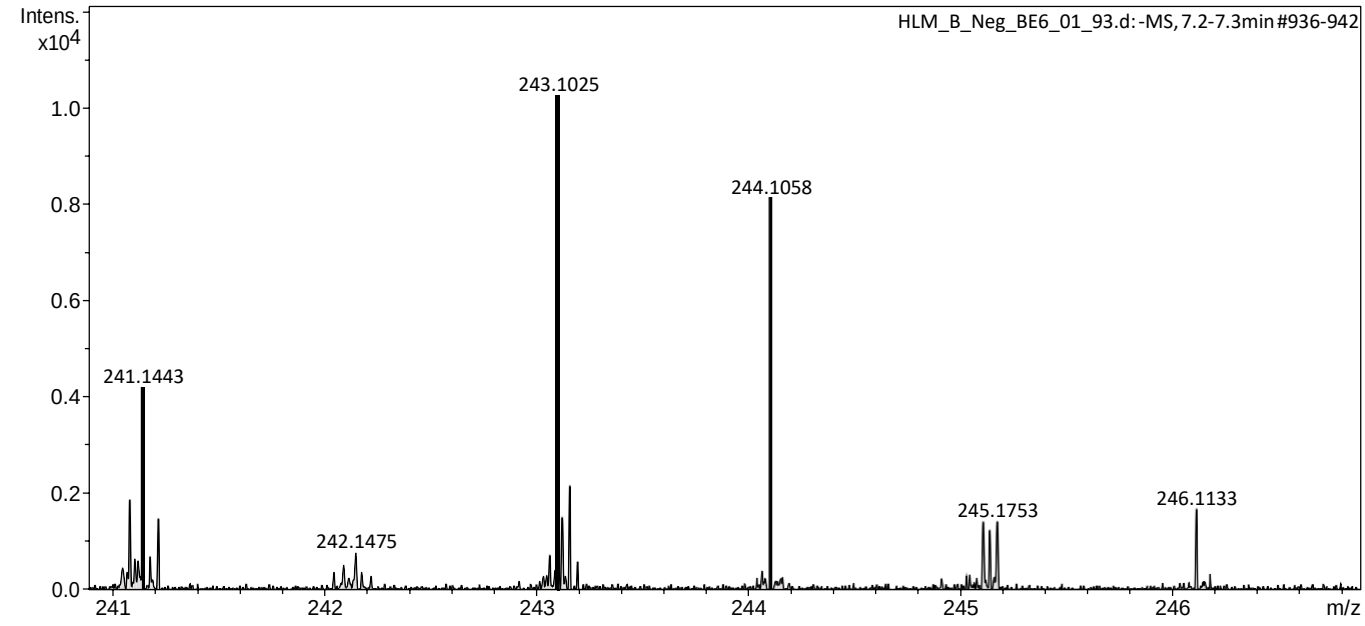
Analysis Info

Acquisition Date	3/25/2016 12:00:08 AM		
Sample Name	HLM_B_Neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





HLM_B_Neg_BE6_01_93.d

Display Report

Analysis Info

Acquisition Date 7/20/2016 7:23:57 PM

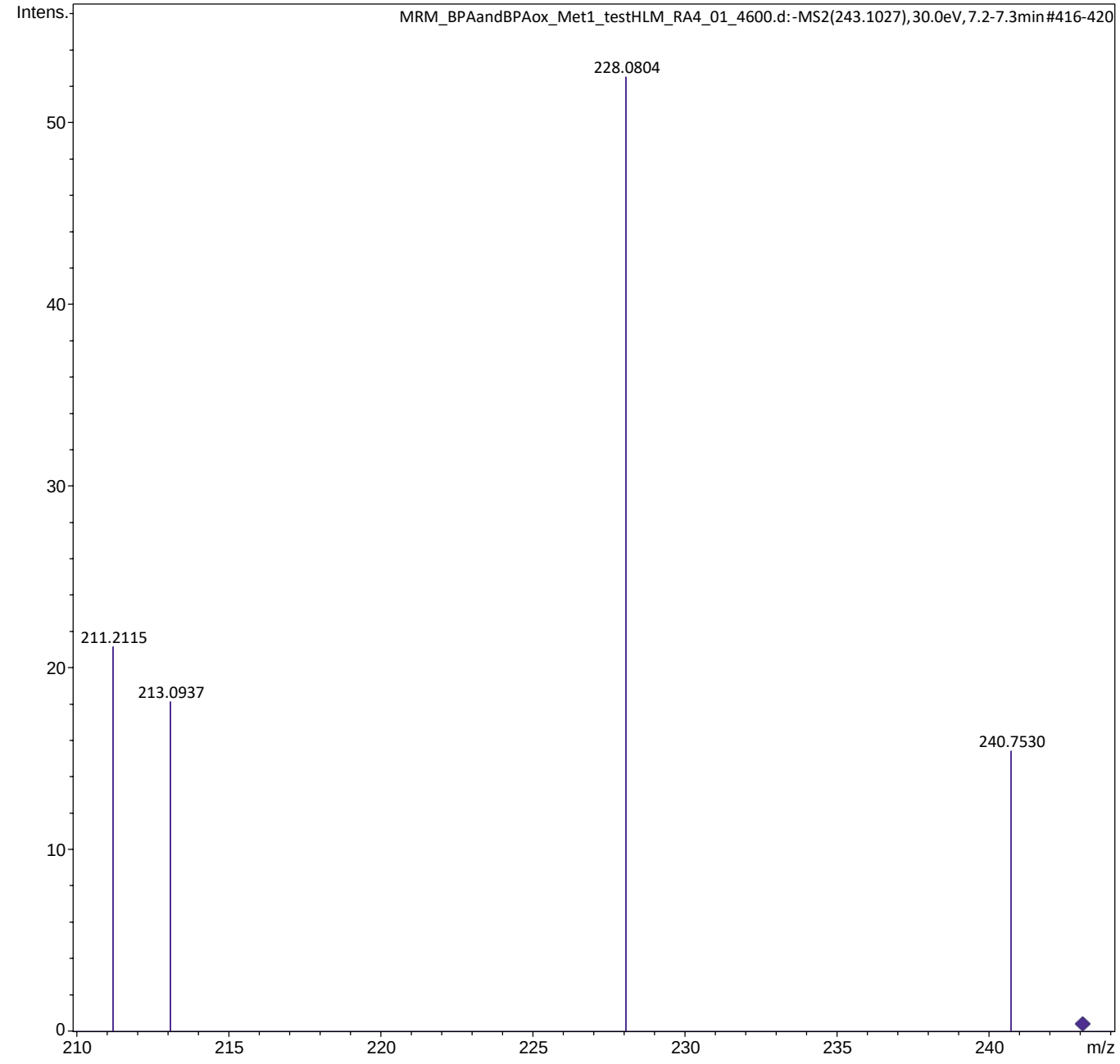
Sample Name MRM_BPAandBPAox_Met1_testHLM

Operator BDAL@DE

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	4.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	12.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C



MRM_BPAandBPAox_Met1_testHLM_RA4_01_4600.d

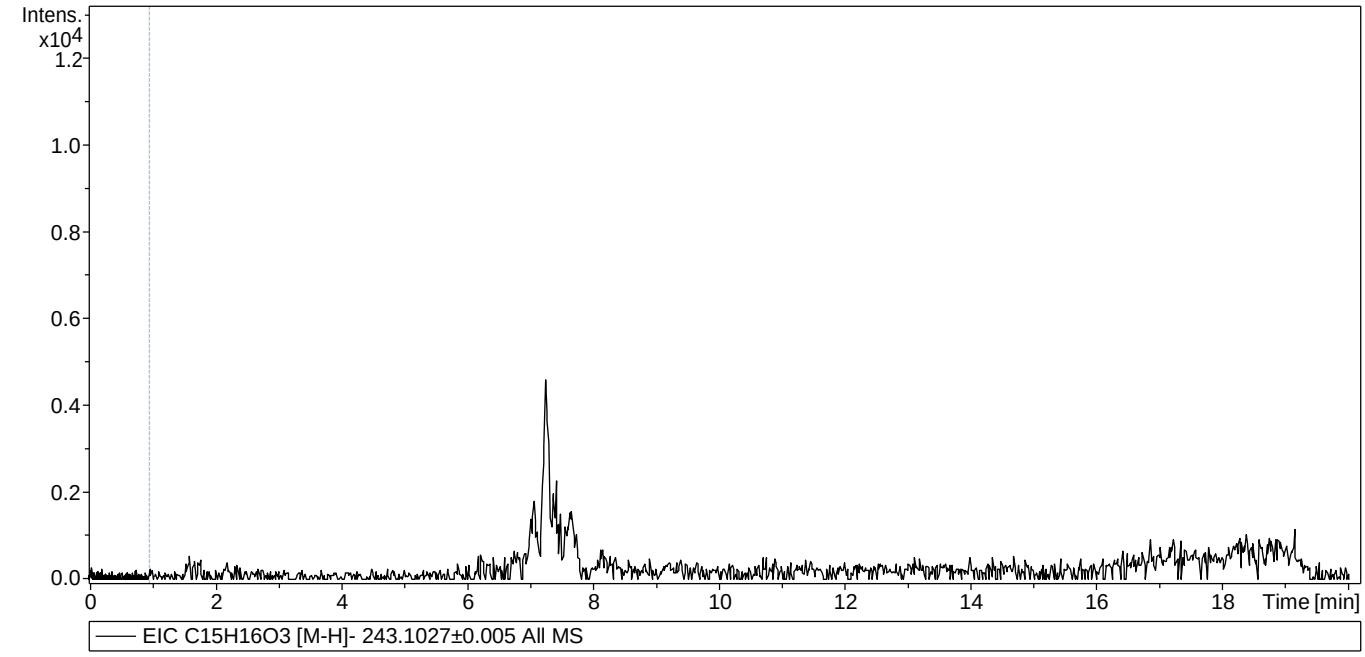
Display Report

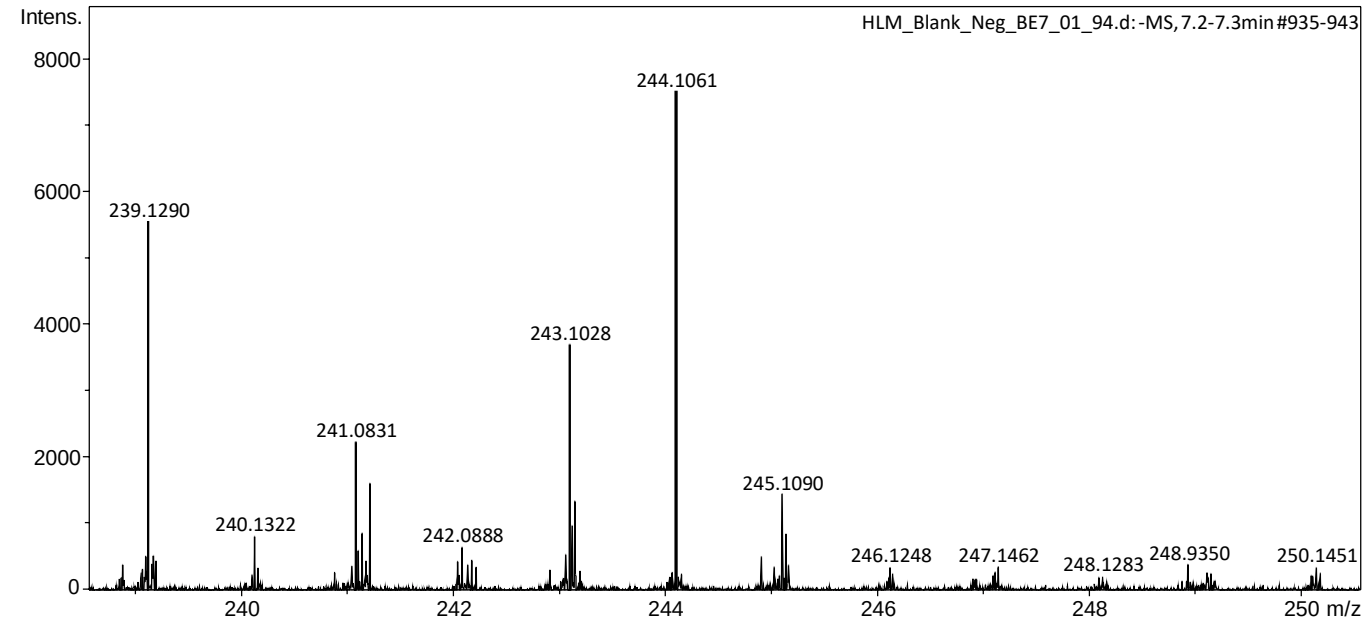
Analysis Info

Acquisition Date	3/25/2016 12:21:20 AM		
Sample Name	HLM_Blank_Neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





HLM_Blank_Neg_BE7_01_94.d

Display Report

Analysis Info

Acquisition Date 11/03/2016 15:39:05

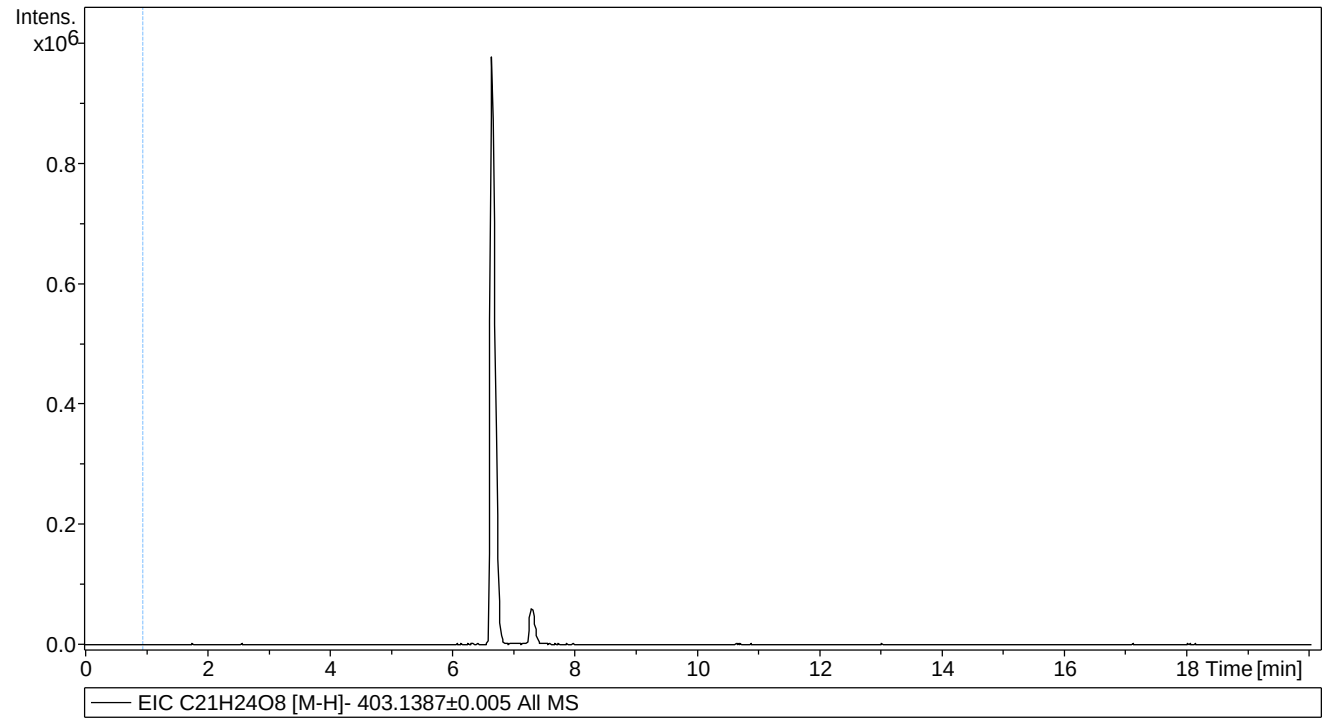
Operator CCAF

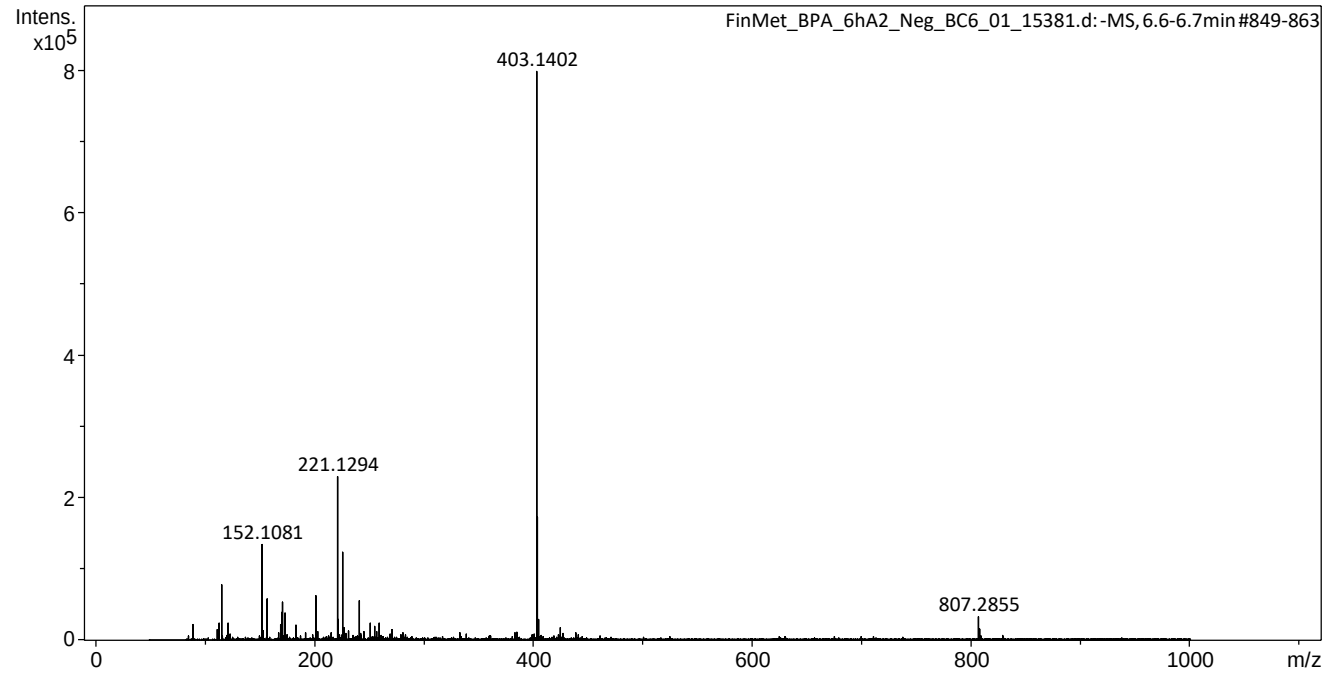
Sample Name FinMet_BPA_6hA2_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





FinMet_BPA_6hA2_Neg_BC6_01_15381.d

Display Report

Analysis Info

Acquisition Date 11/03/2016 15:39:05

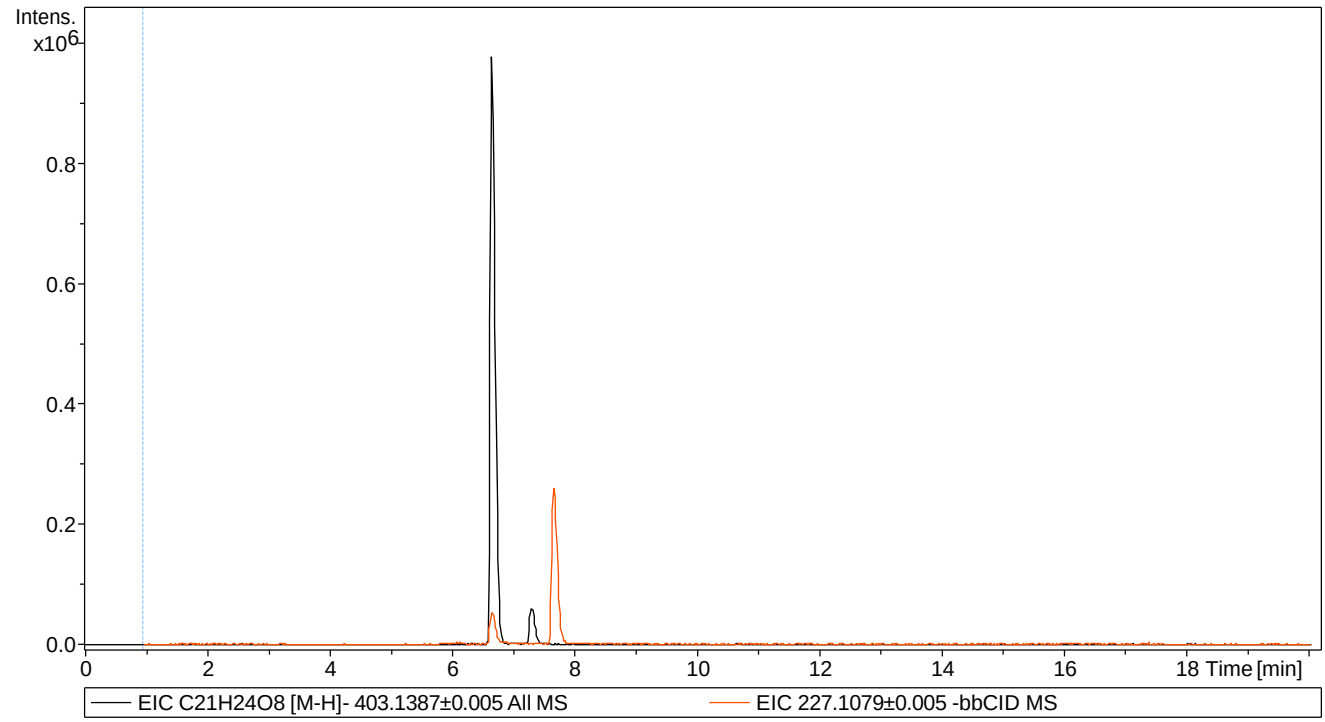
Operator CCAF

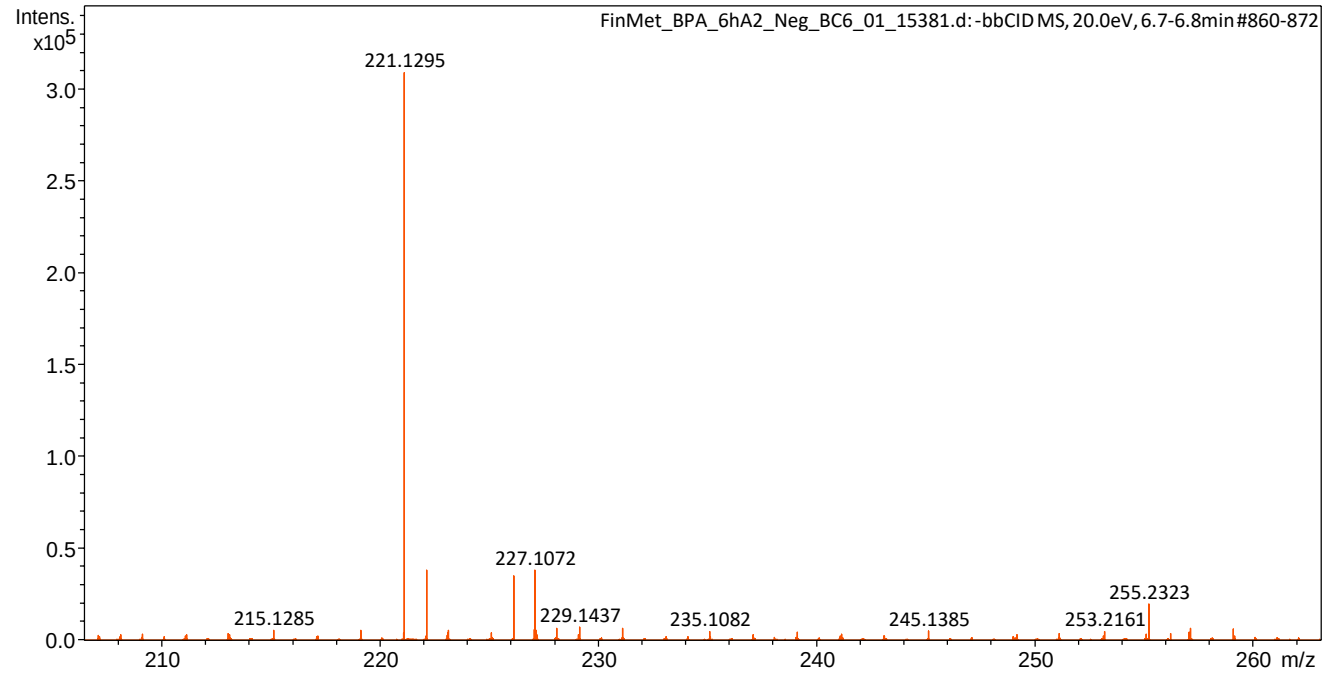
Sample Name FinMet_BPA_6hA2_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





FinMet_BPA_6hA2_Neg_BC6_01_15381.d

Display Report

Analysis Info

Acquisition Date 11/03/2016 19:12:24

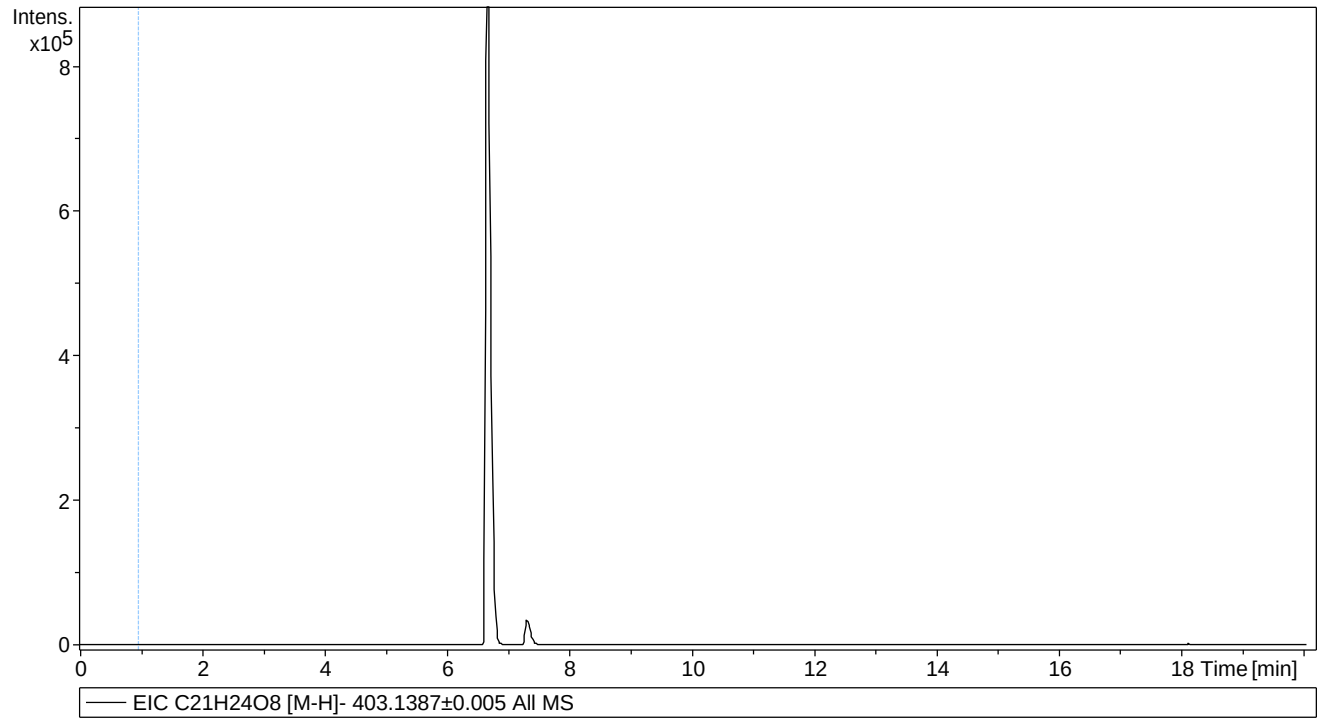
Operator CCAF

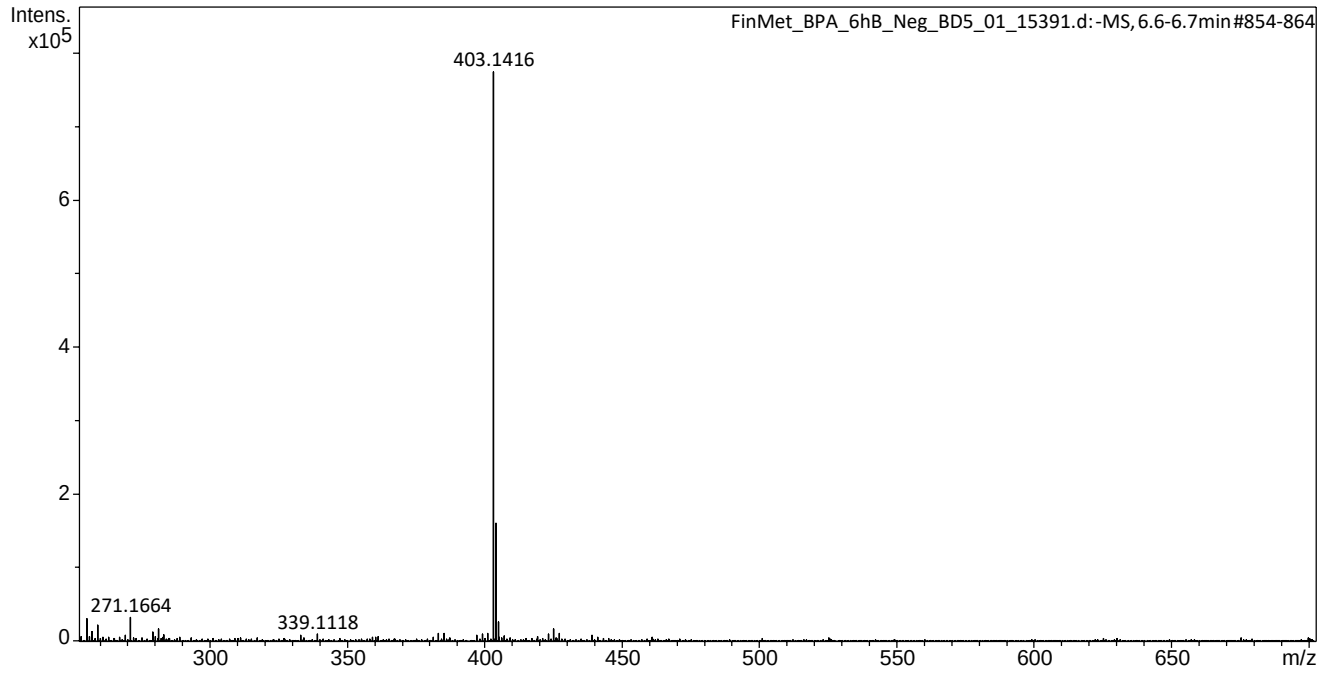
Sample Name FinMet_BPA_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 11/03/2016 19:12:24

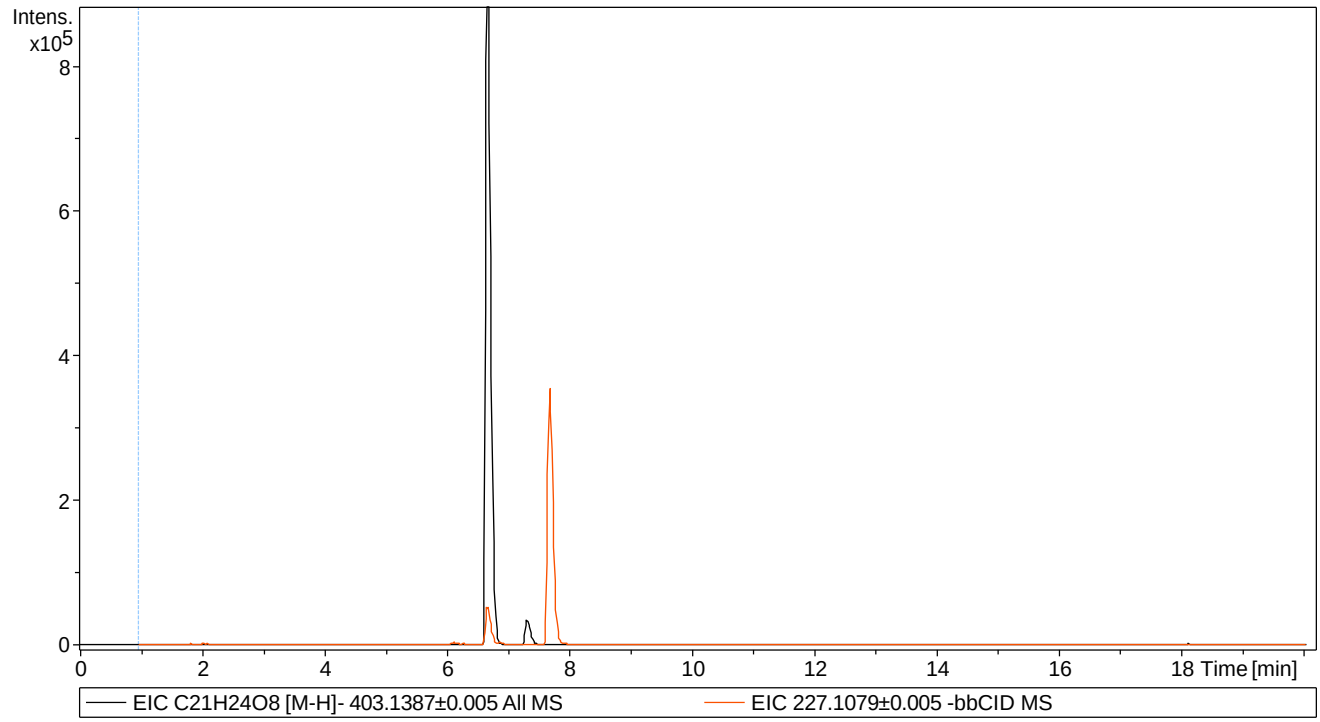
Operator CCAF

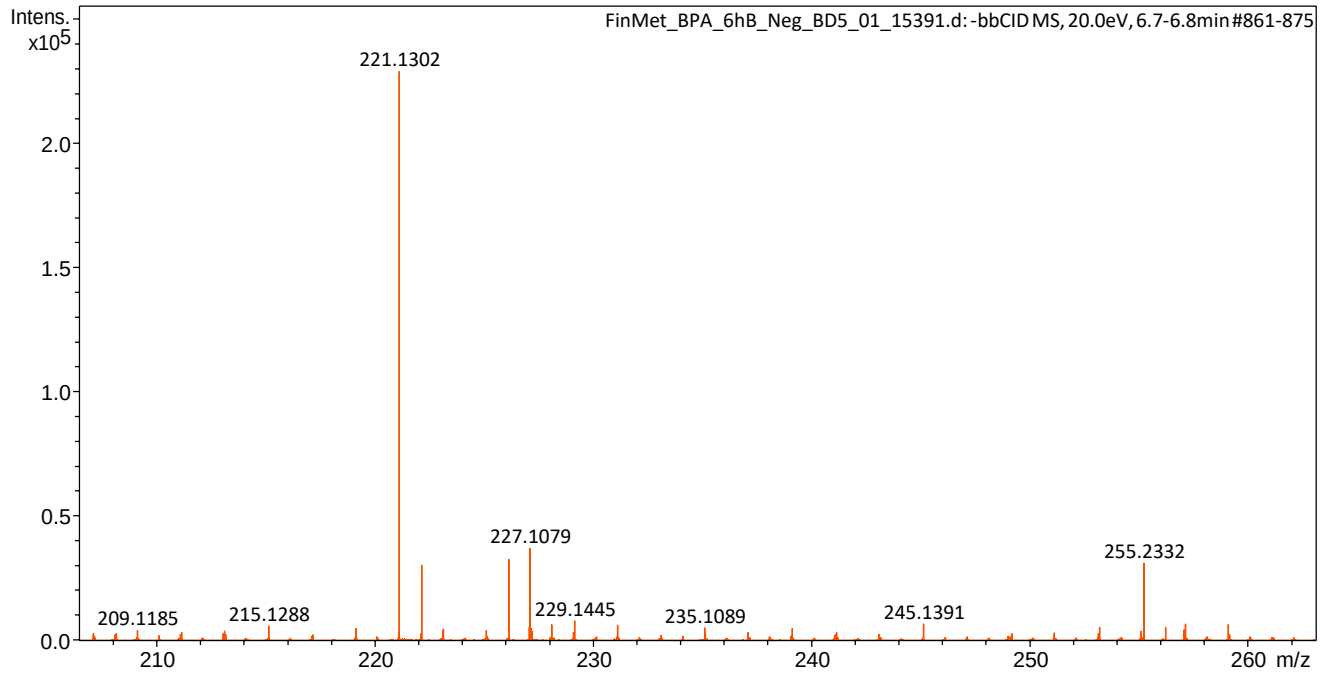
Sample Name FinMet_BPA_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 11/03/2016 22:24:37

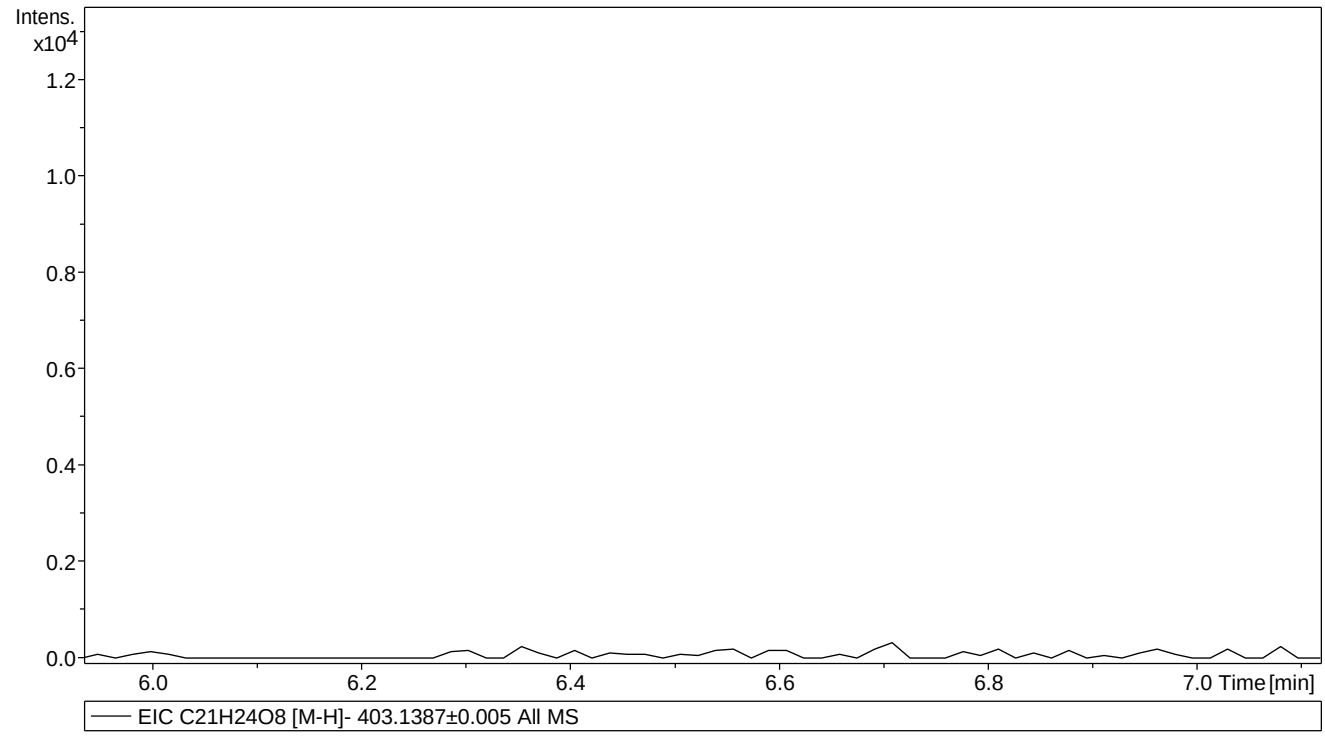
Operator CCAF

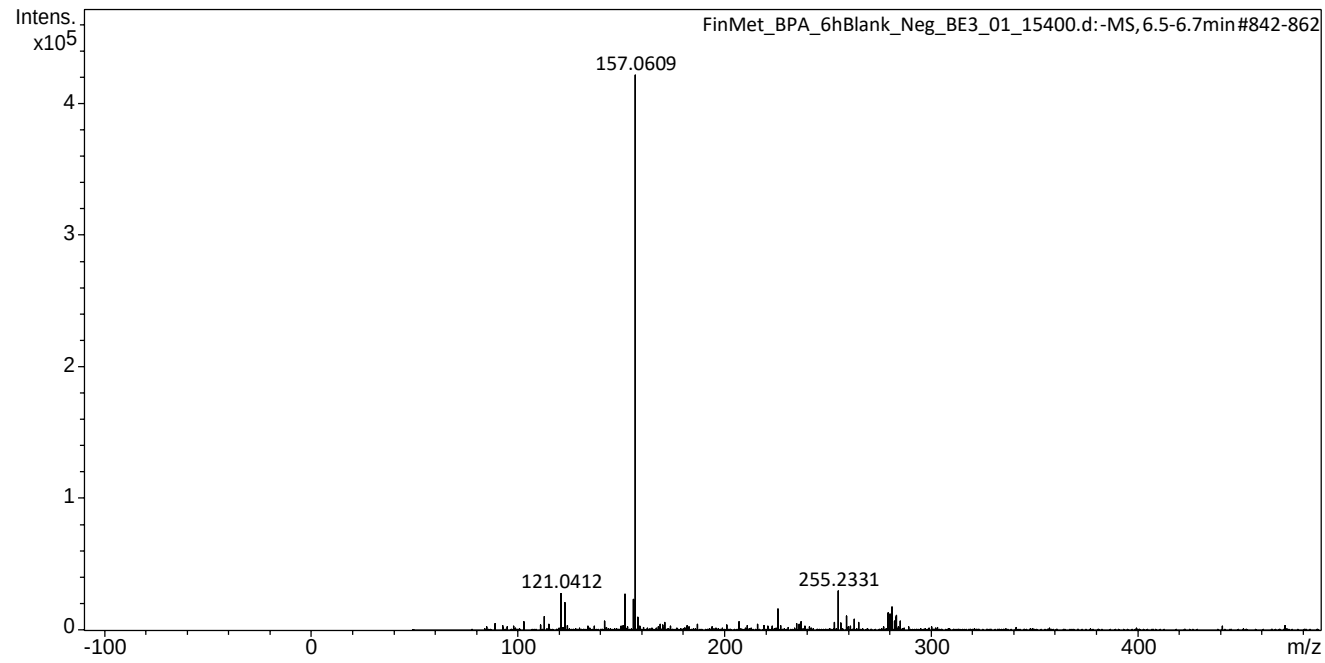
Sample Name FinMet_BPA_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





FinMet_BPA_6hBlank_Neg_BE3_01_15400.d

Display Report

Analysis Info

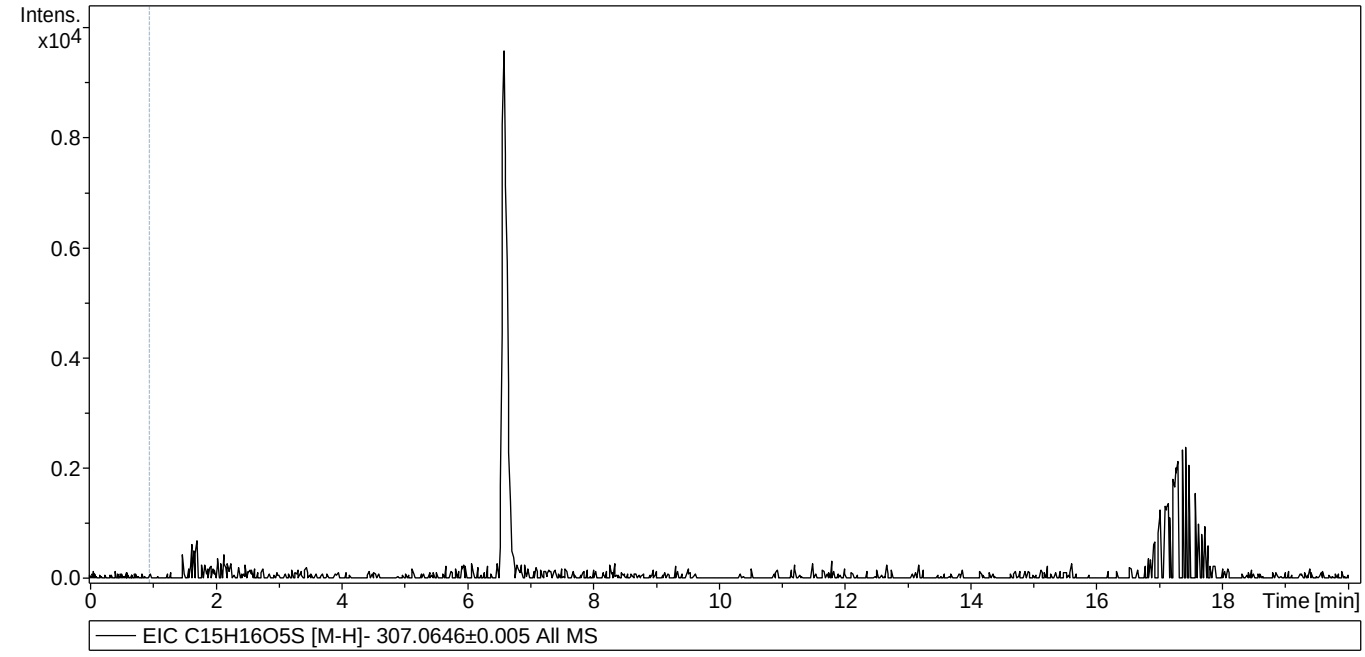
Acquisition Date 1/13/2016 8:11:34 PM

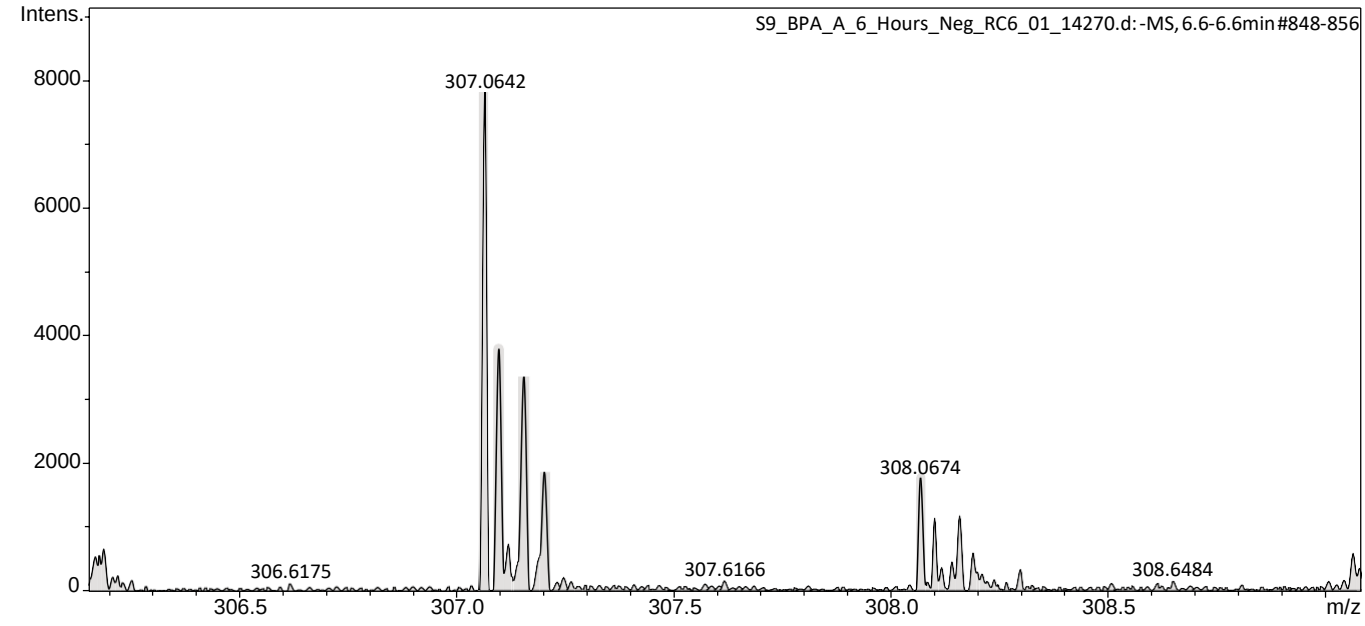
Sample Name S9_BPA_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_BPA_A_6_Hours_Neg_RC6_01_14270.d

Display Report

Analysis Info

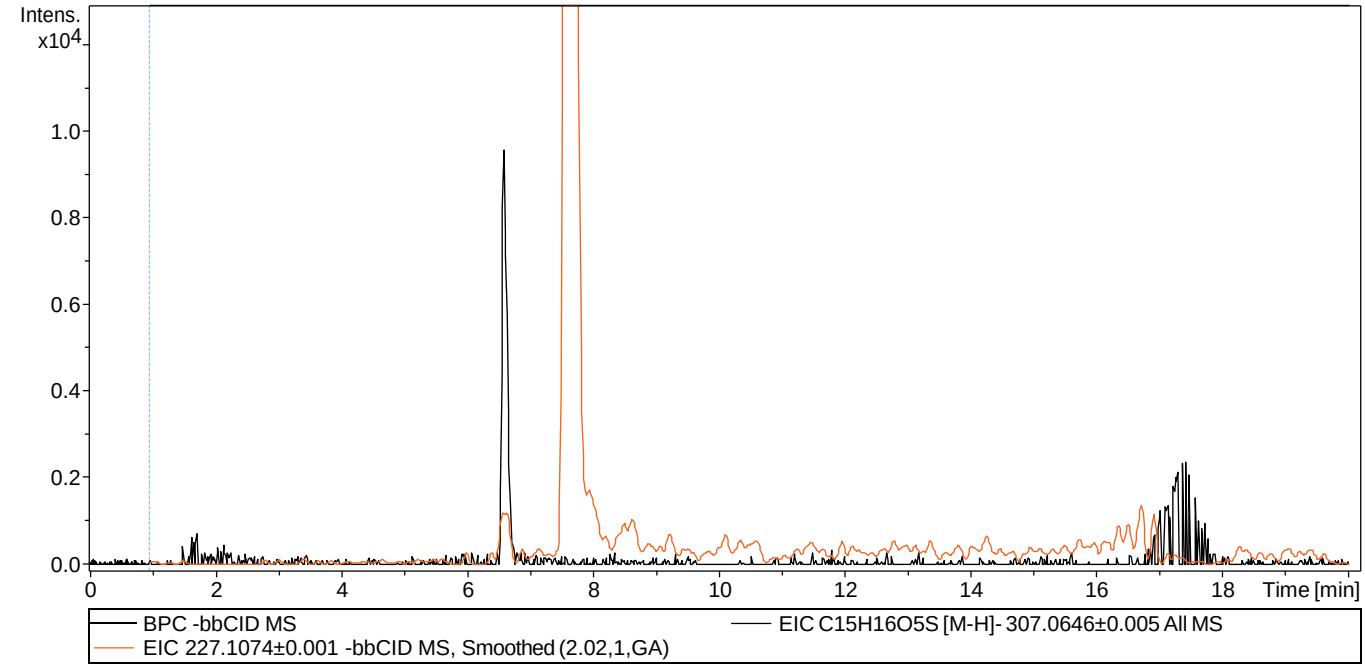
Acquisition Date 1/13/2016 8:11:34 PM

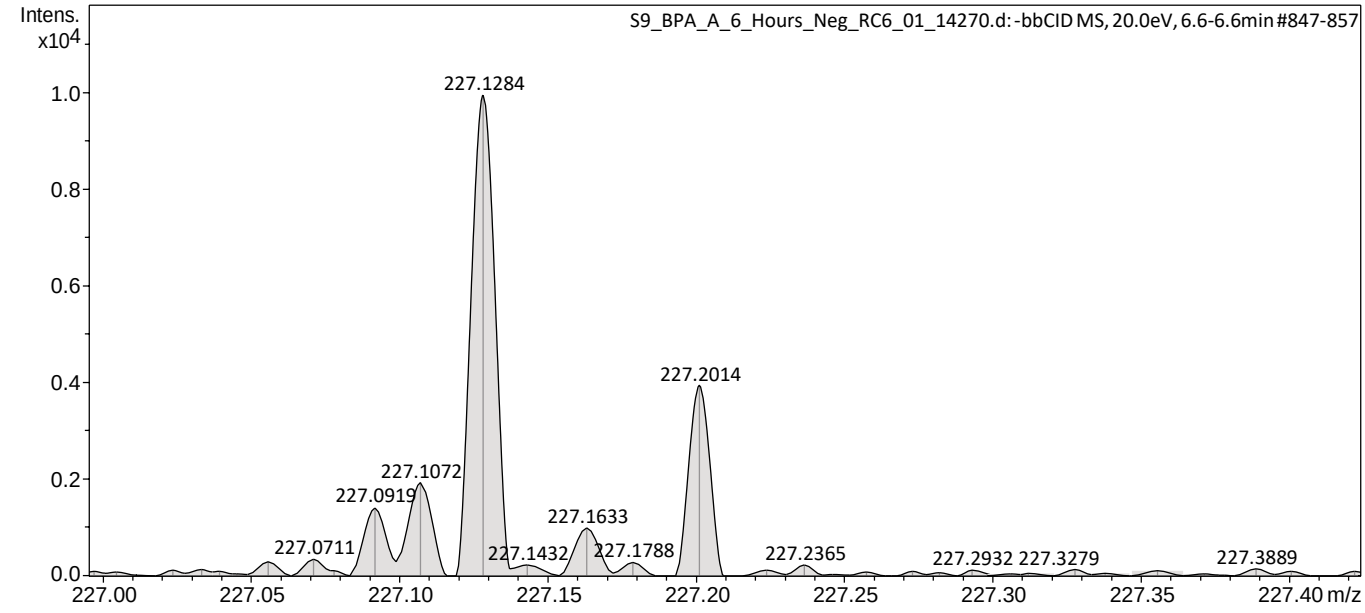
Sample Name S9_BPA_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

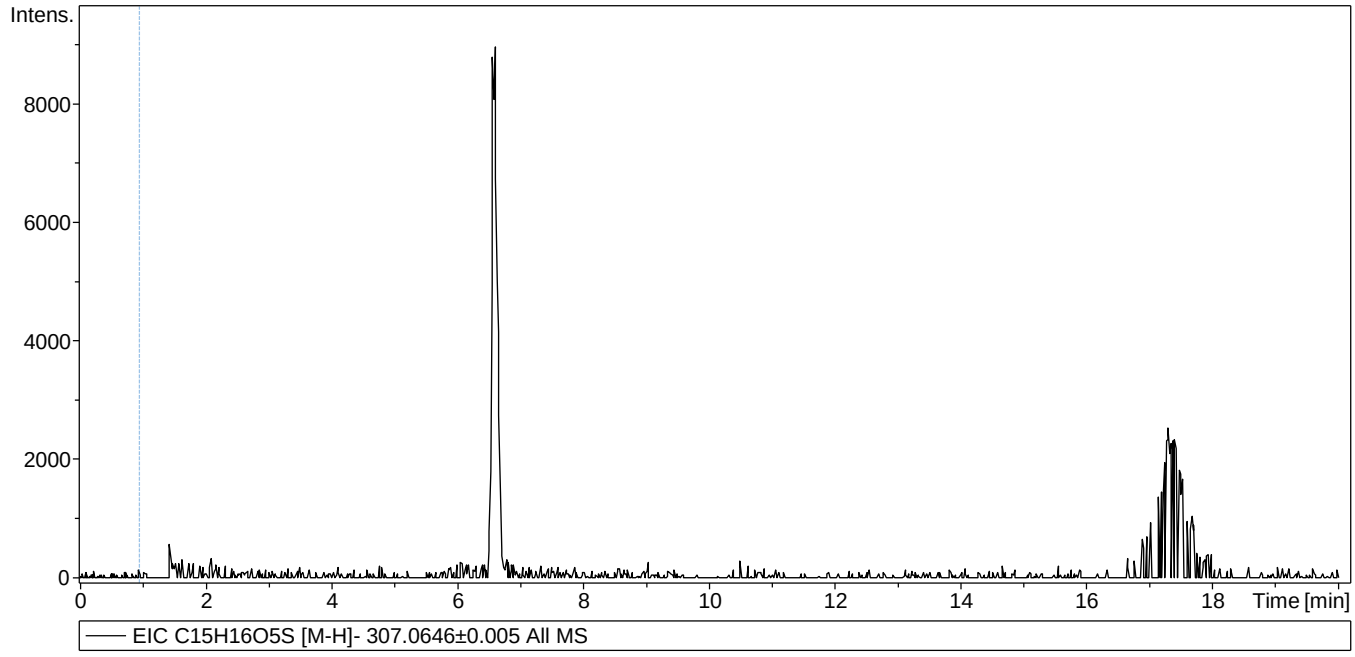
Acquisition Date 1/13/2016 10:19:14 PM

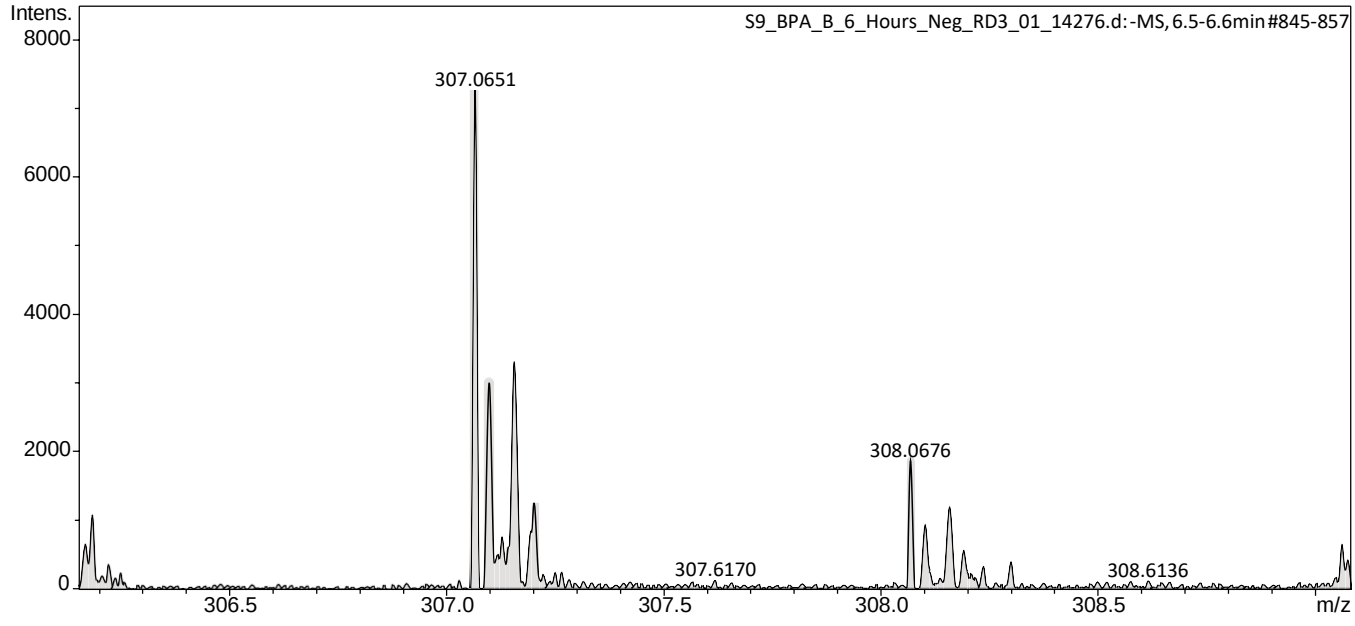
Sample Name S9_BPA_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_BPA_B_6_Hours_Neg_RD3_01_14276.d

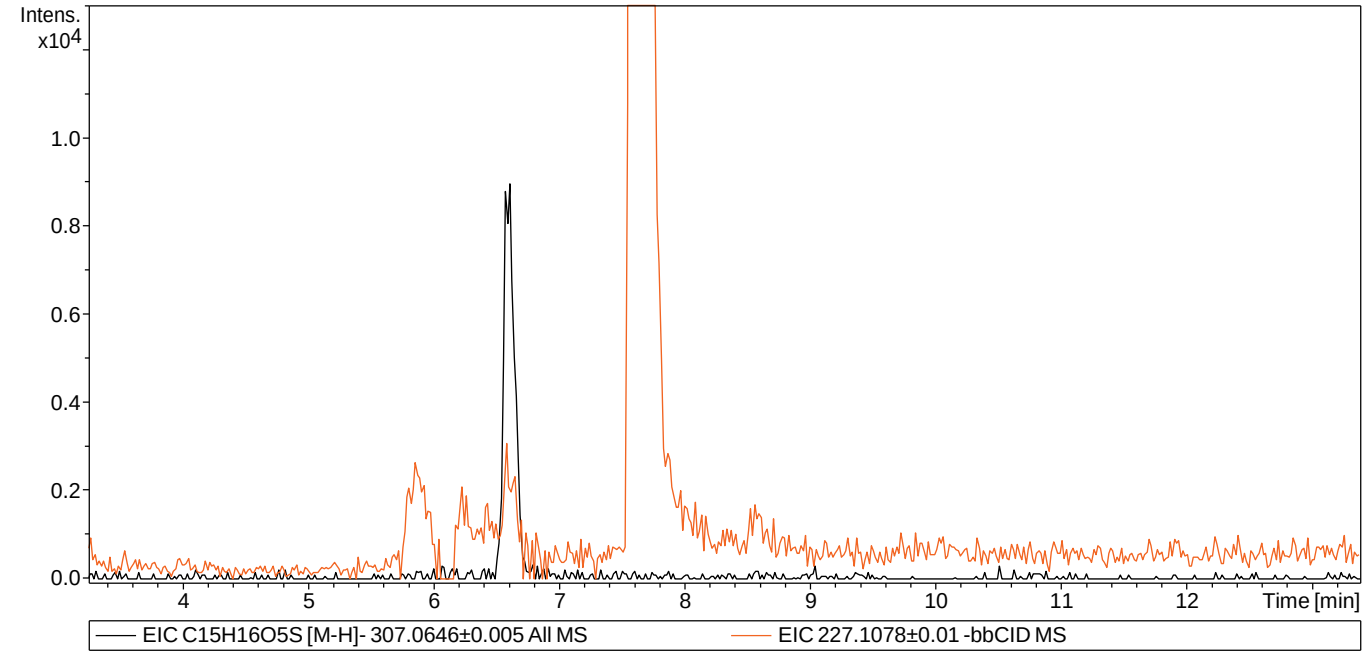
Display Report

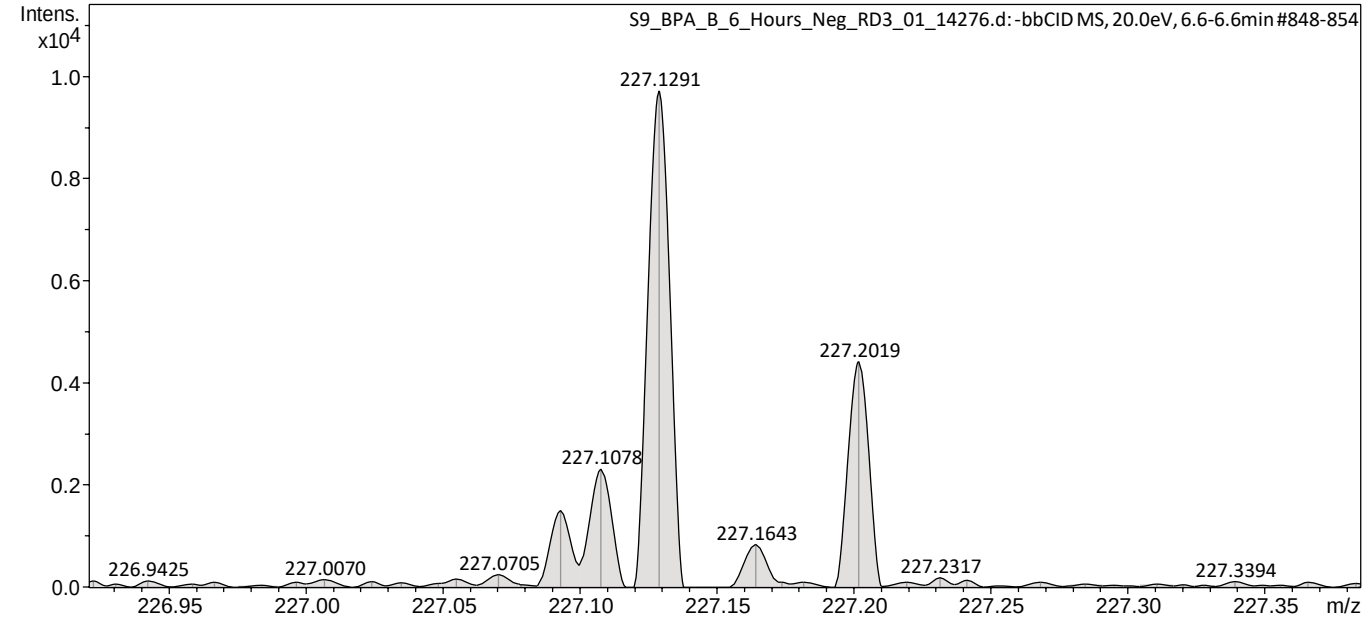
Analysis Info

Acquisition Date	1/13/2016 10:19:14 PM		
Sample Name	S9_BPA_B_6_Hours_Neg	Operator	CCAF
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_BPA_B_6_Hours_Neg_RD3_01_14276.d

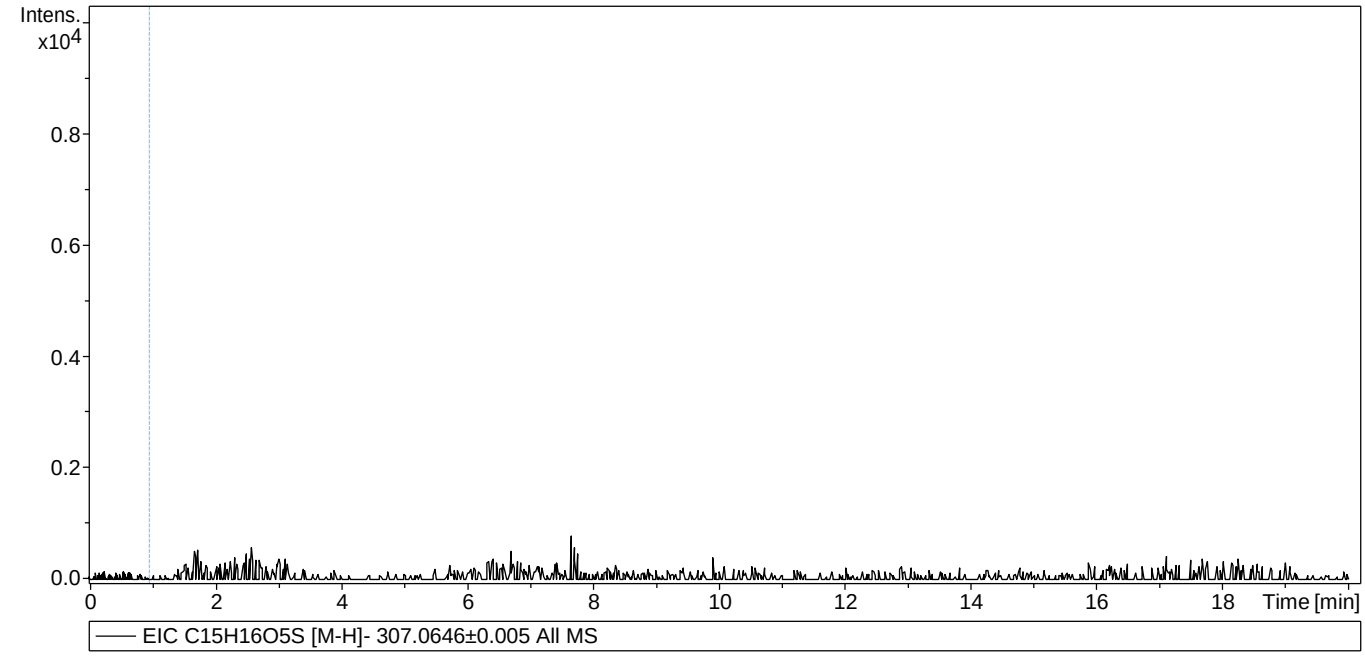
Display Report

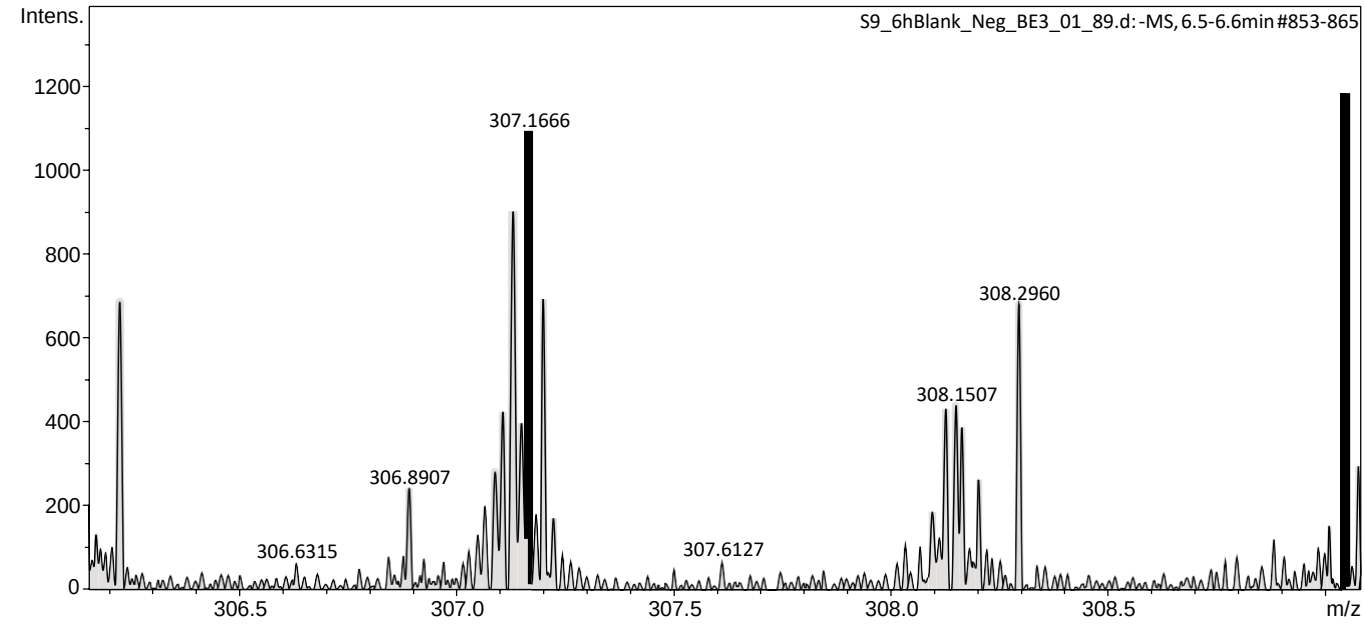
Analysis Info

Acquisition Date	3/24/2016 10:35:20 PM		
Sample Name	S9_6hBlank_Neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_6hBlank_Neg_BE3_01_89.d

Display Report

Analysis Info

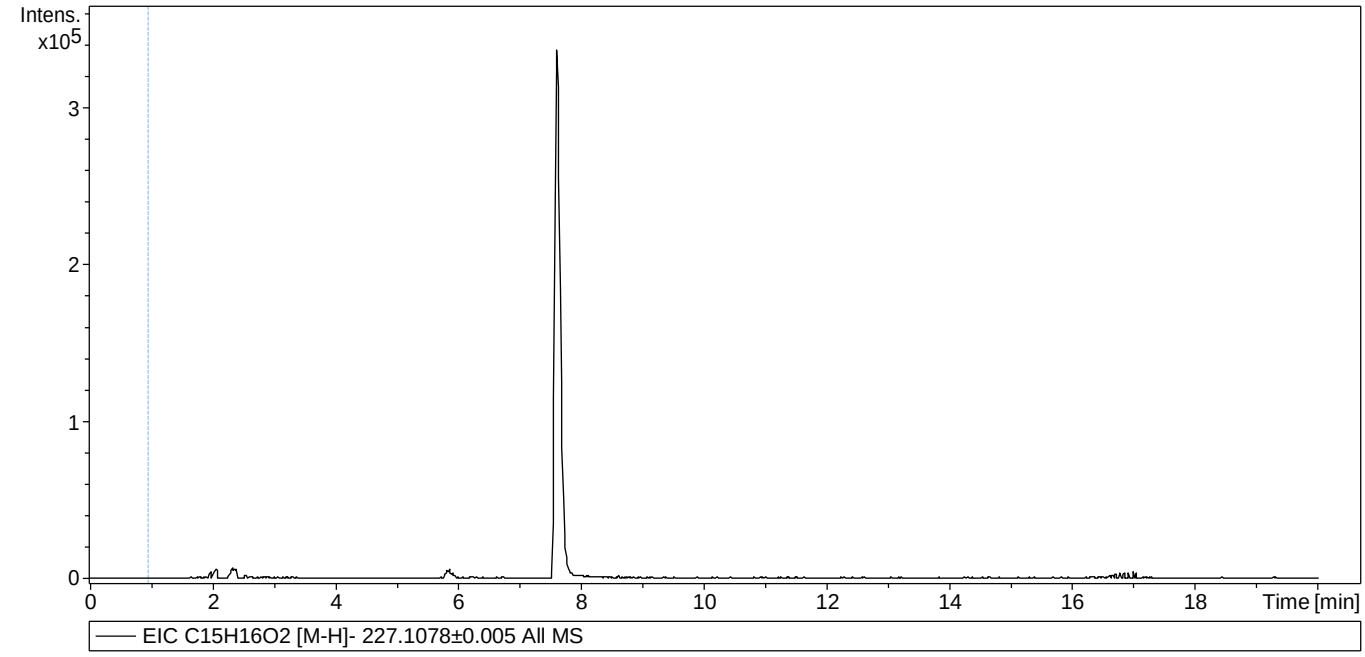
Acquisition Date 1/13/2016 8:11:34 PM

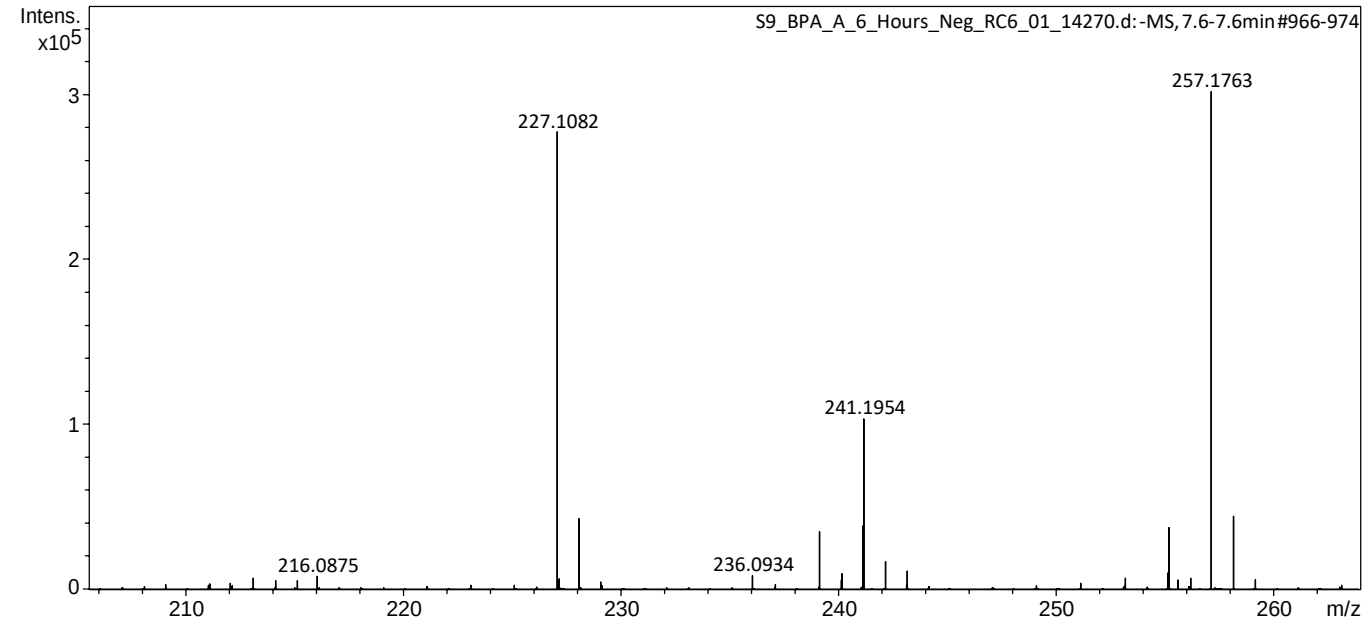
Sample Name S9_BPA_A_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_BPA_A_6_Hours_Neg_RC6_01_14270.d

Display Report

Analysis Info

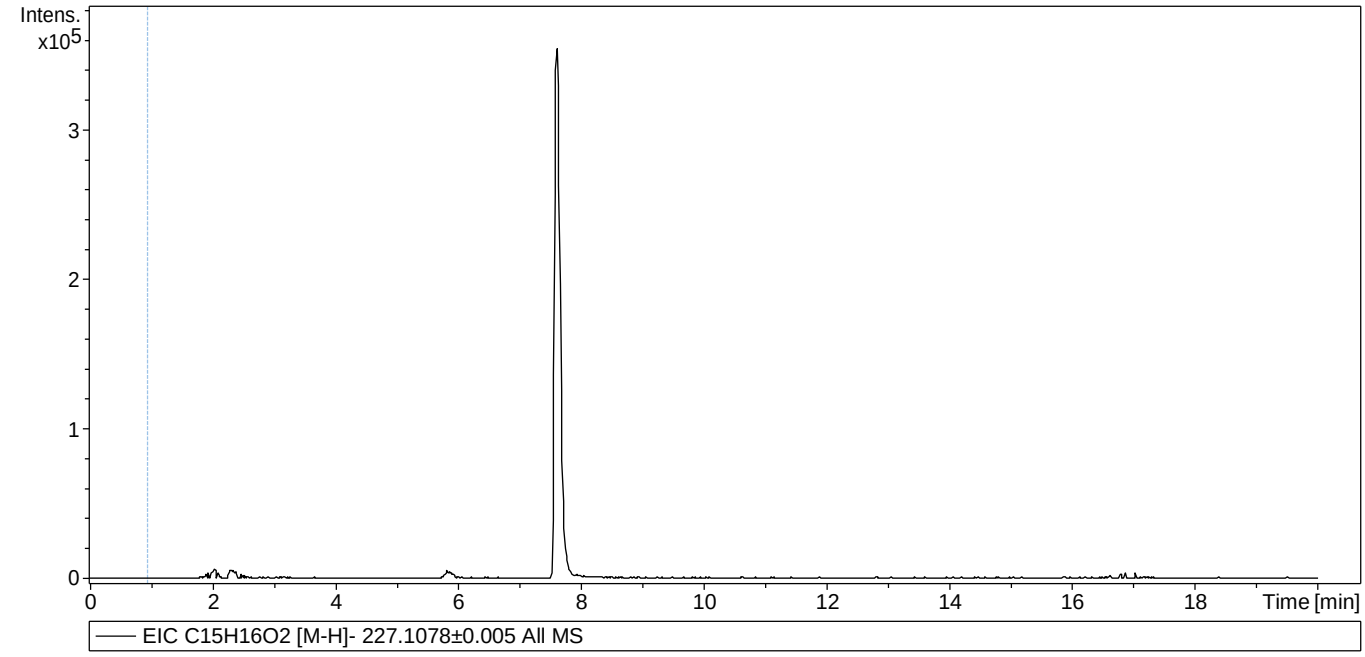
Acquisition Date 1/13/2016 10:19:14 PM

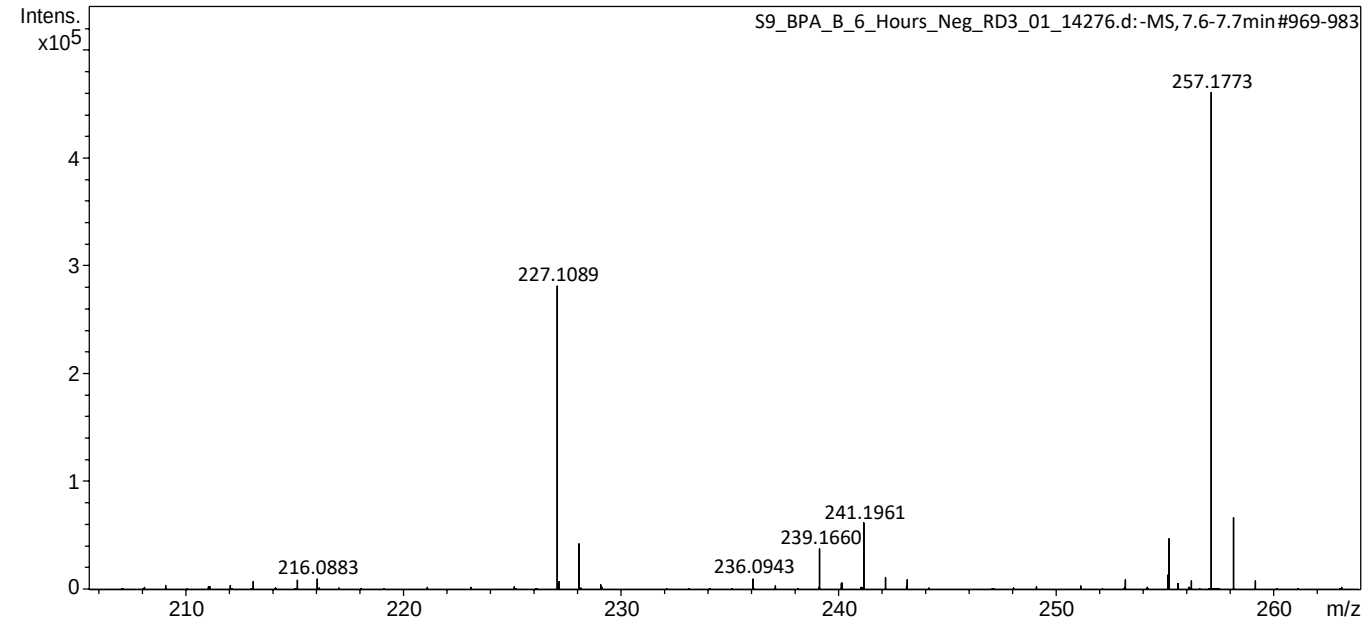
Sample Name S9_BPA_B_6_Hours_Neg

Operator CCAF
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_BPA_B_6_Hours_Neg_RD3_01_14276.d

Display Report

Analysis Info

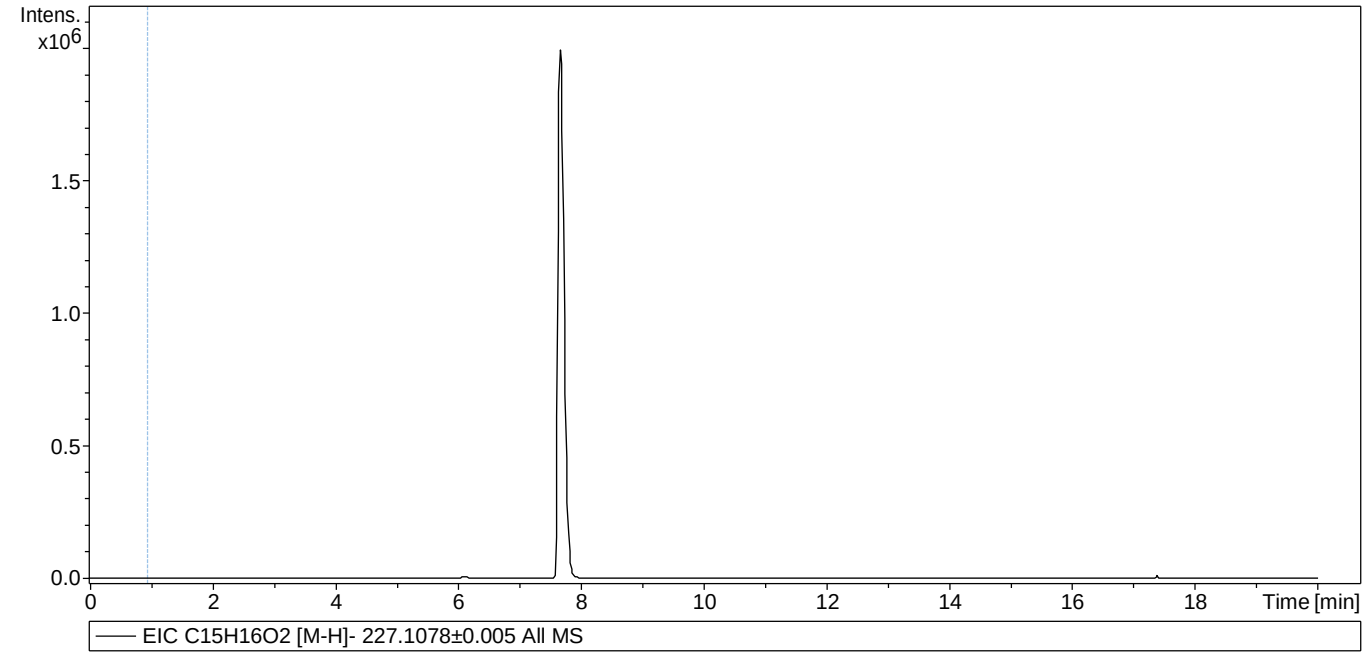
Acquisition Date 3/24/2016 10:35:20 PM

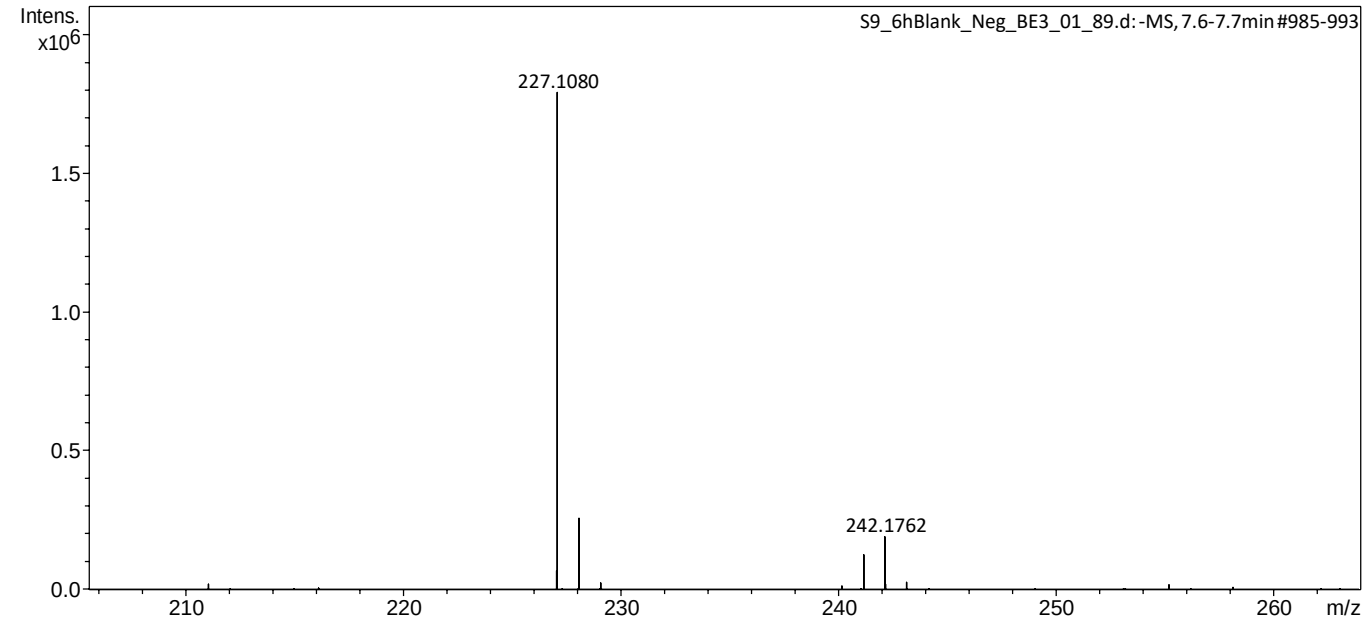
Sample Name S9_6hBlank_Neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





S9_6hBlank_Neg_BE3_01_89.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

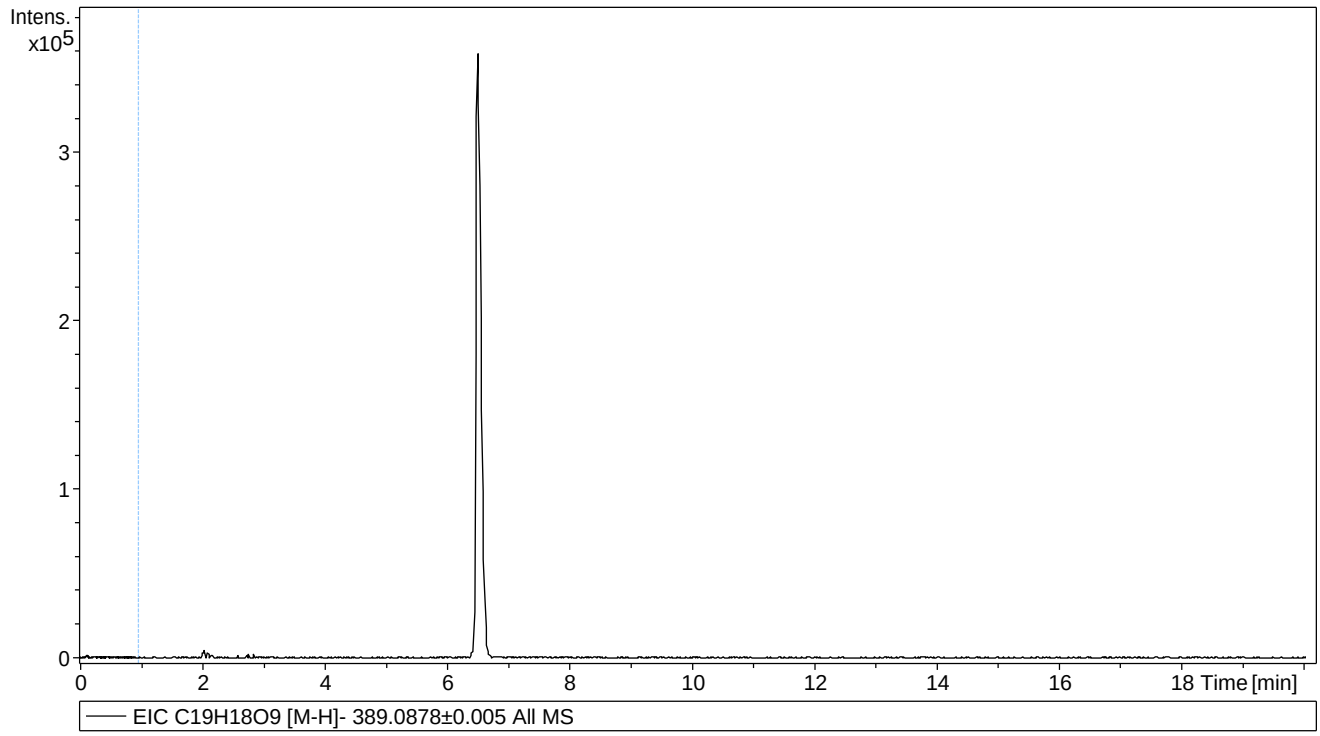
Operator BDAL@DE

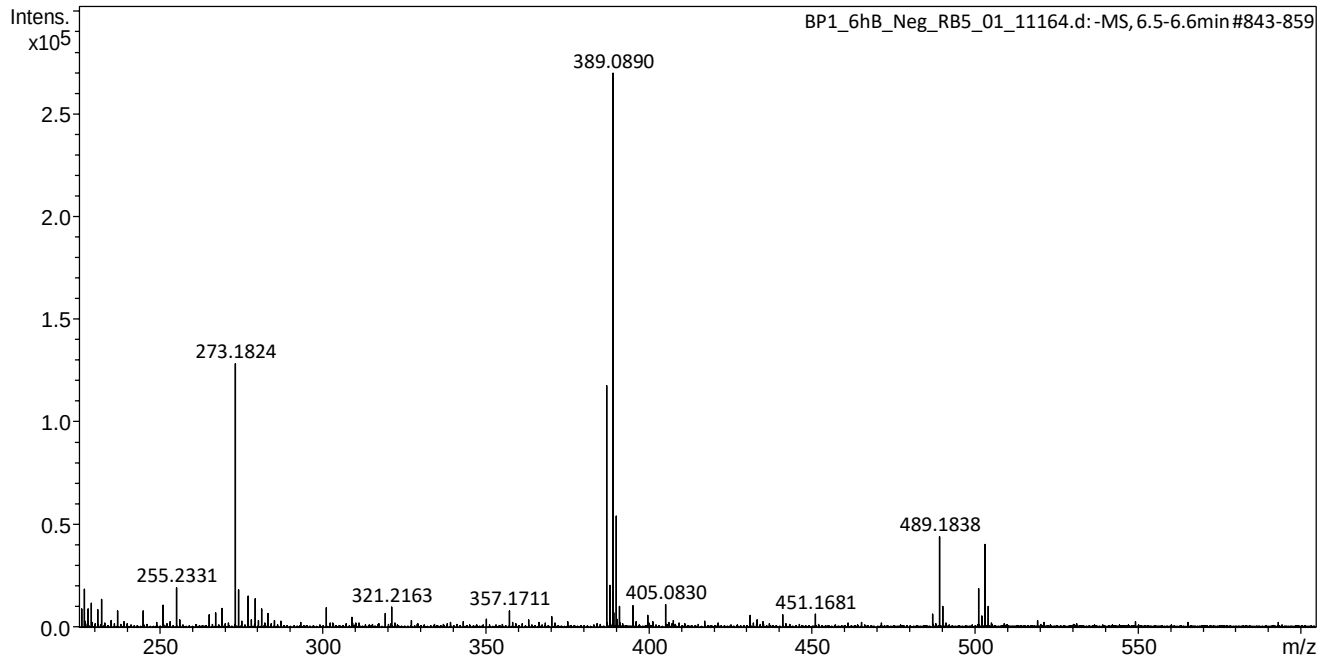
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hB_Neg_RB5_01_11164.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

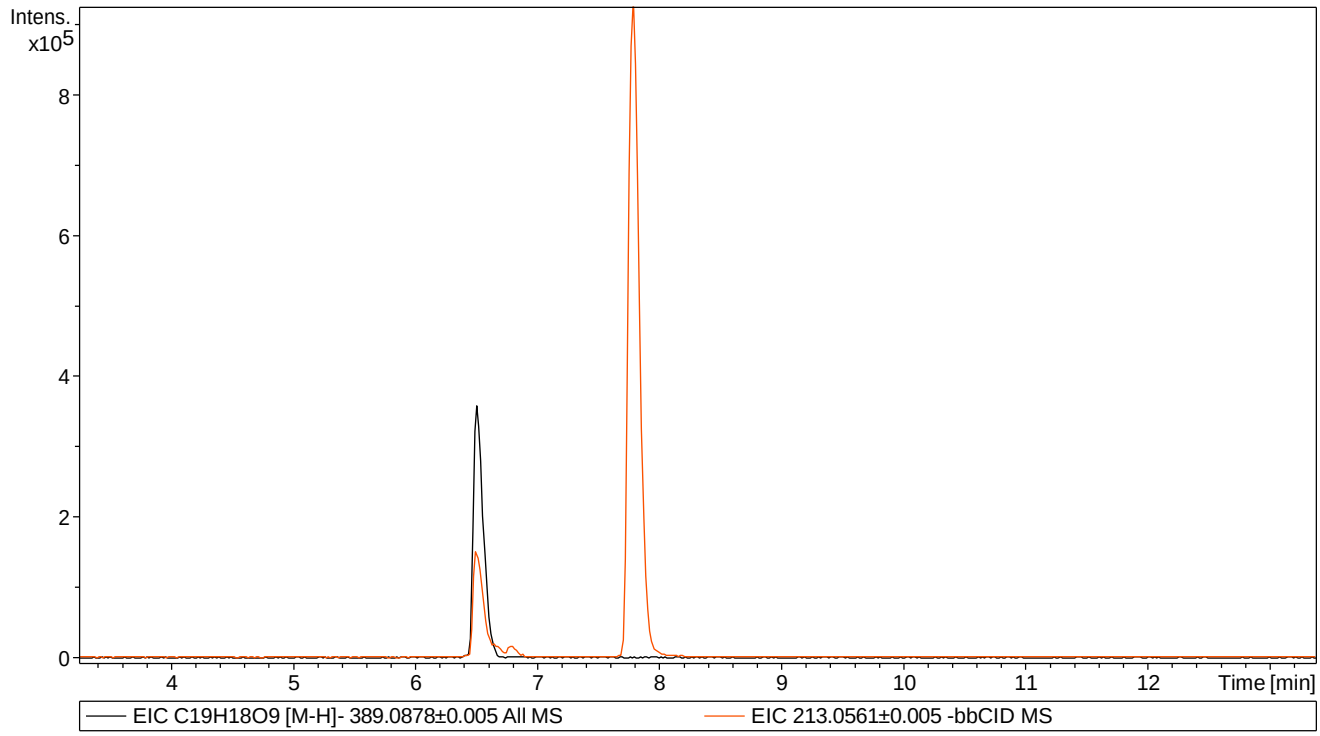
Operator BDAL@DE

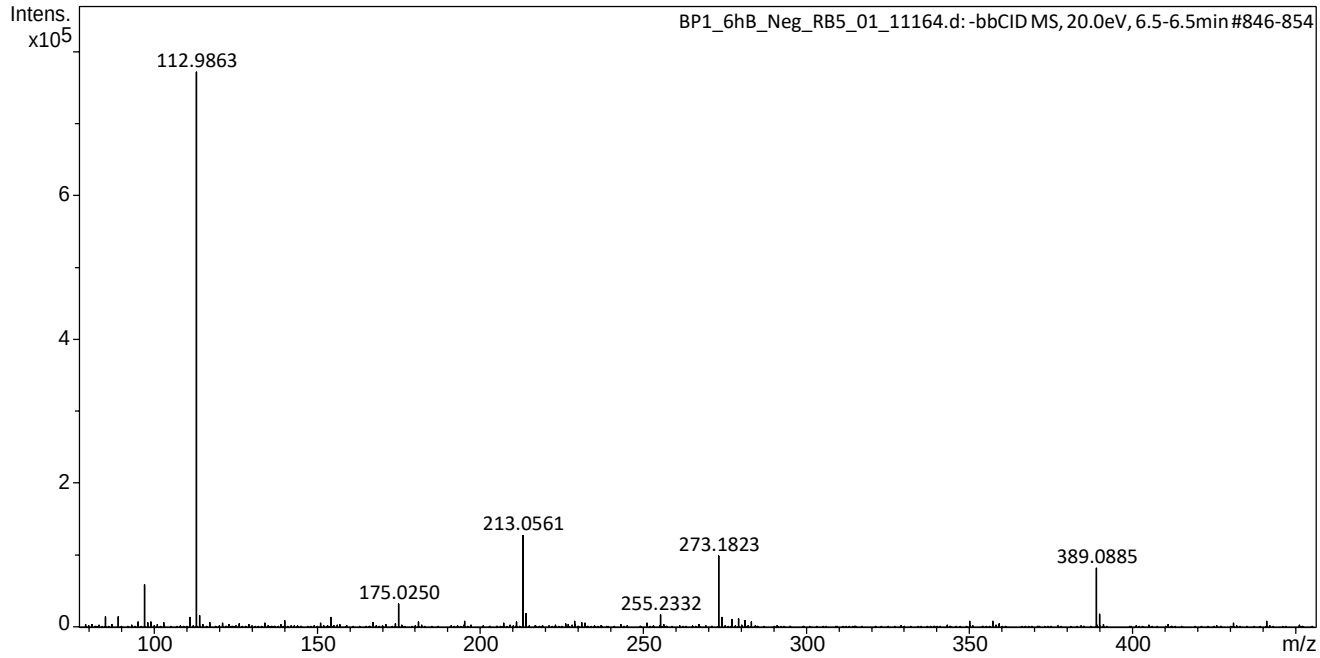
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

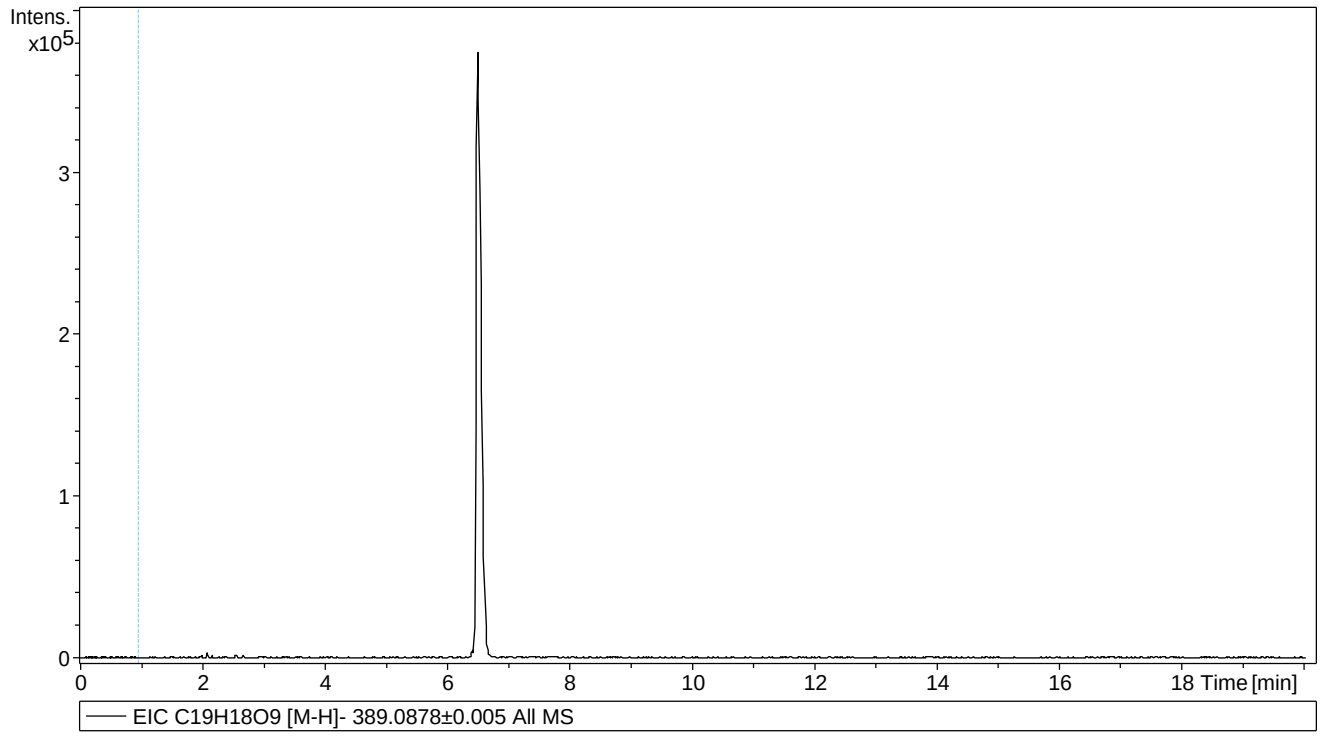
Operator BDAL@DE

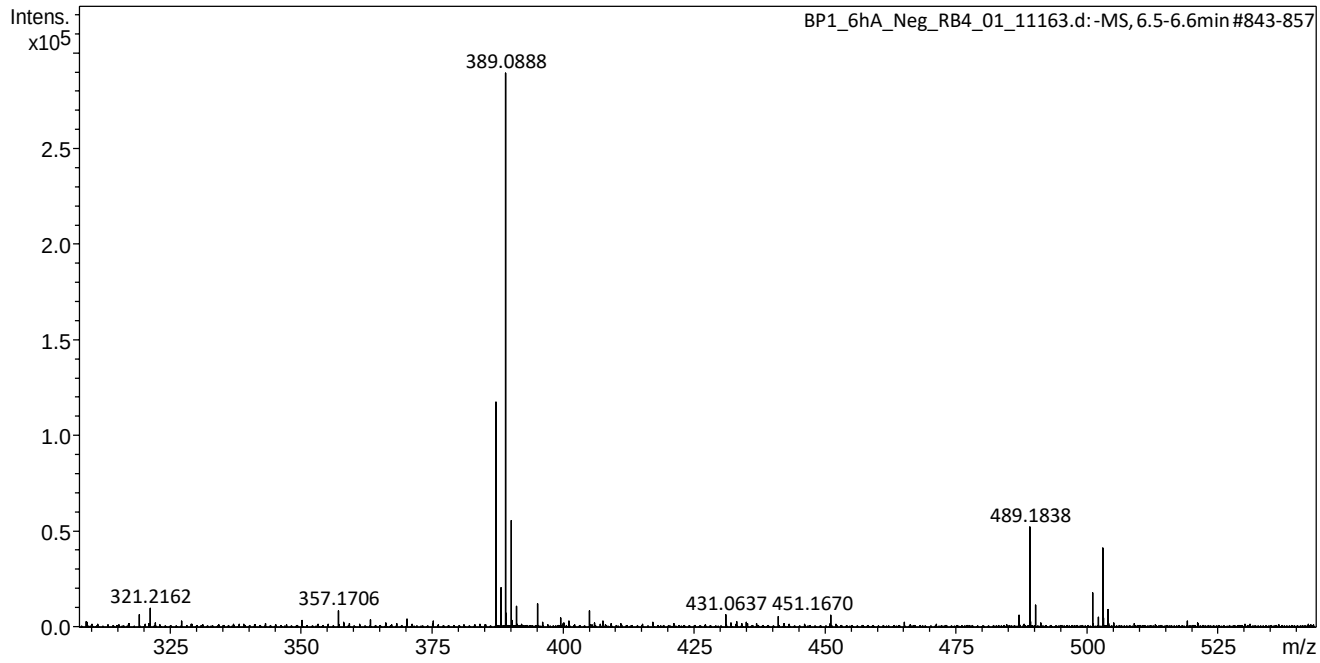
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

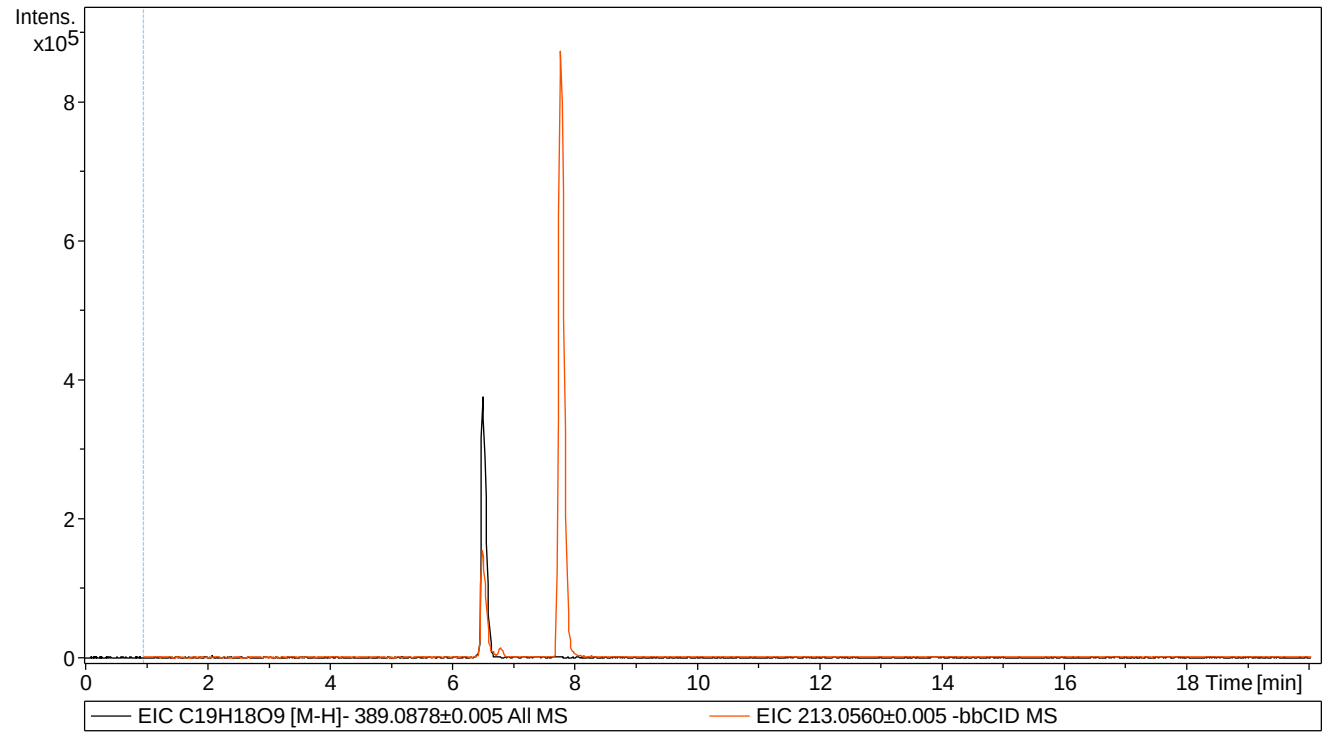
Operator BDAL@DE

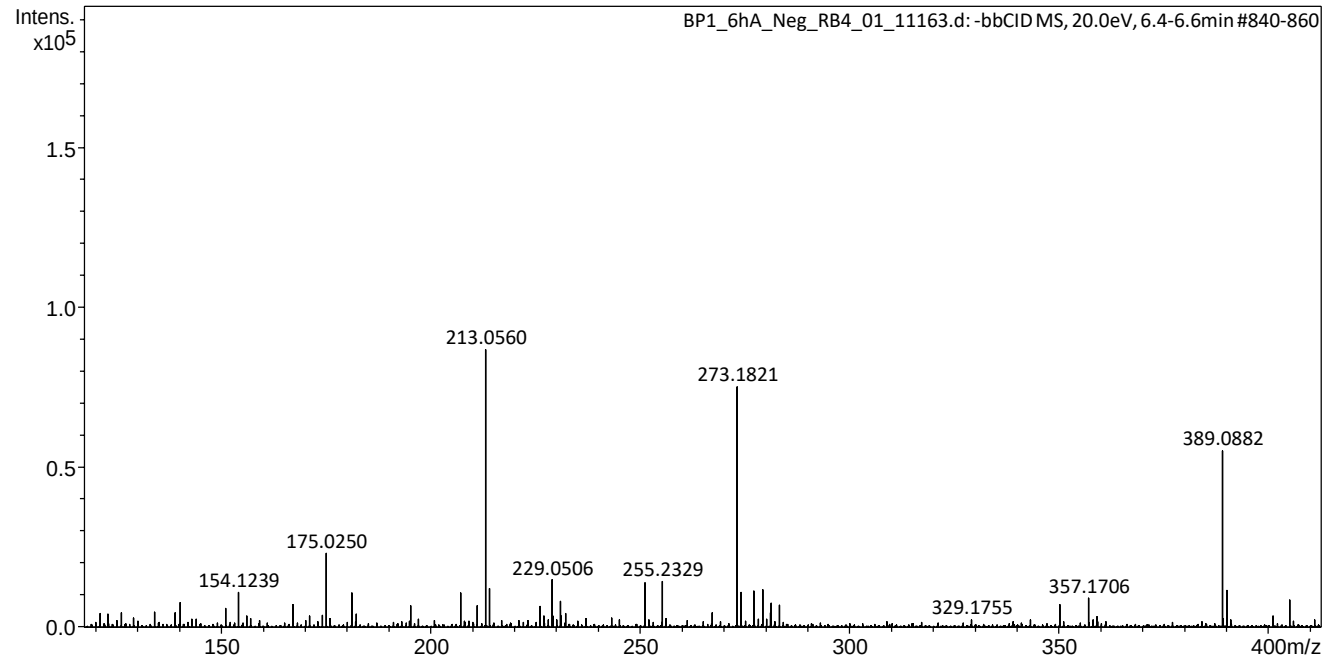
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hA_Neg_RB4_01_11163.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

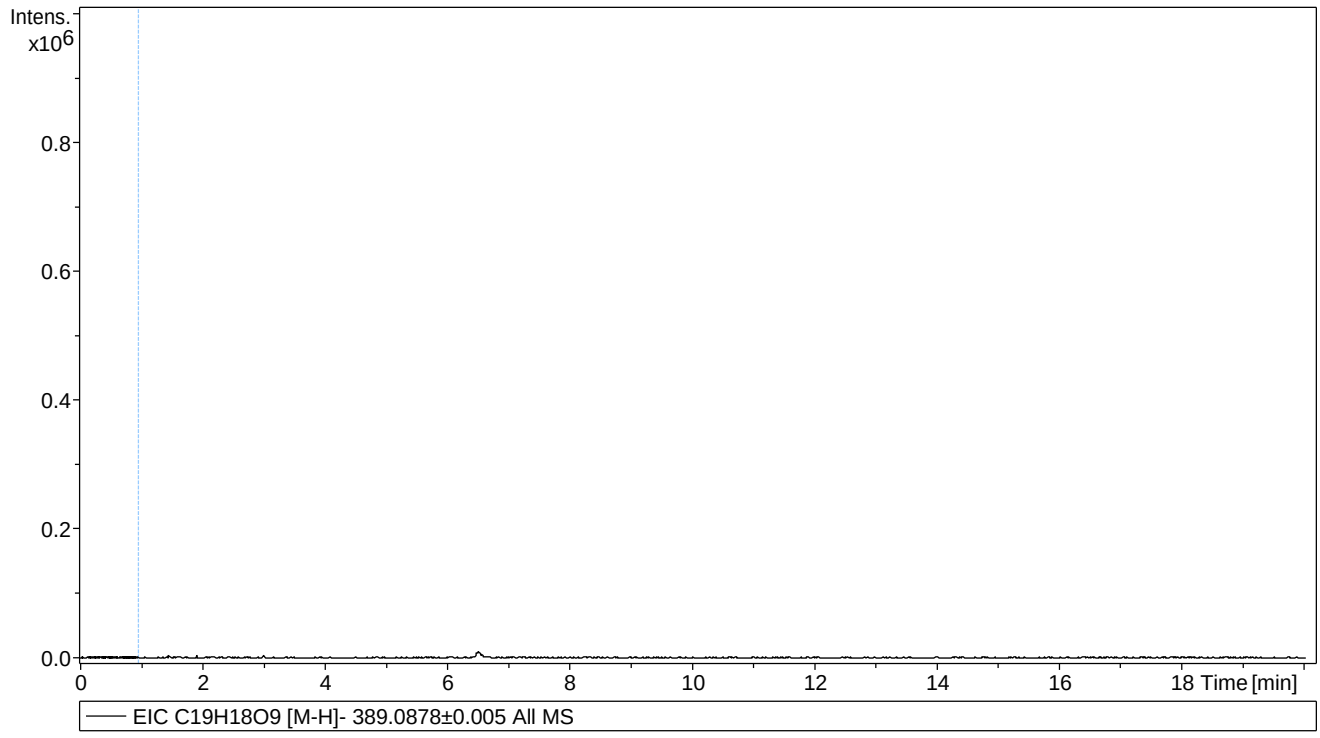
Operator BDAL@DE

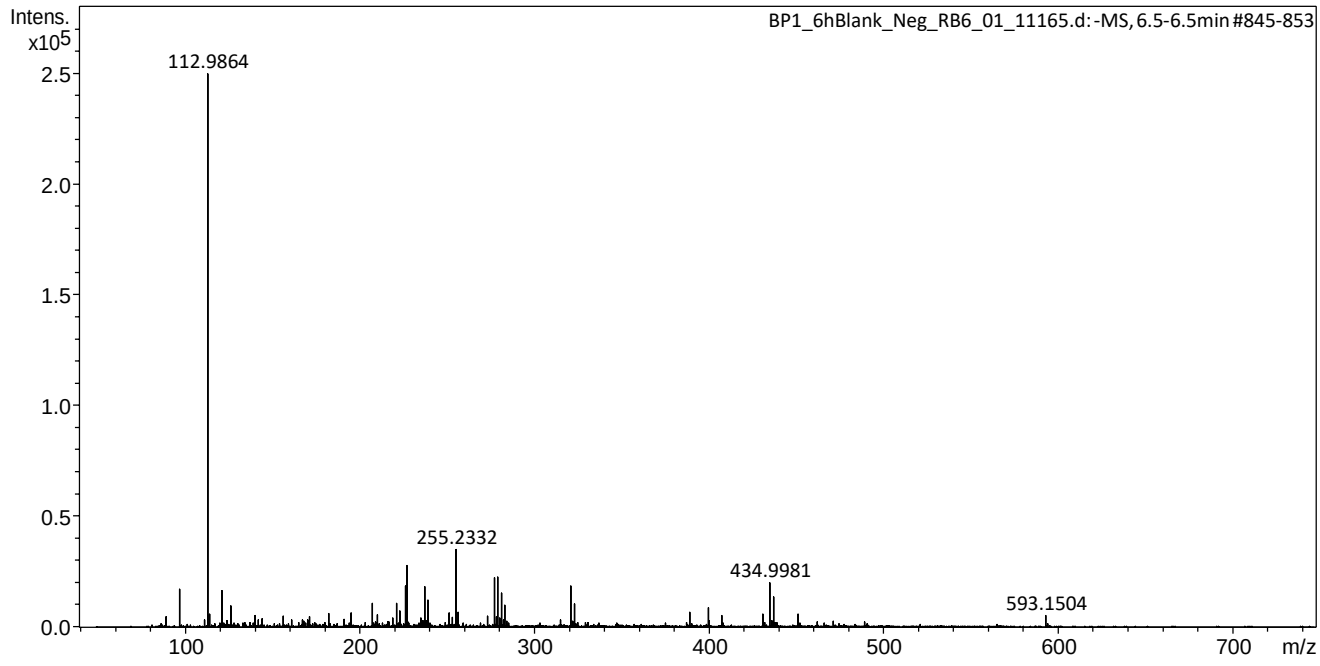
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

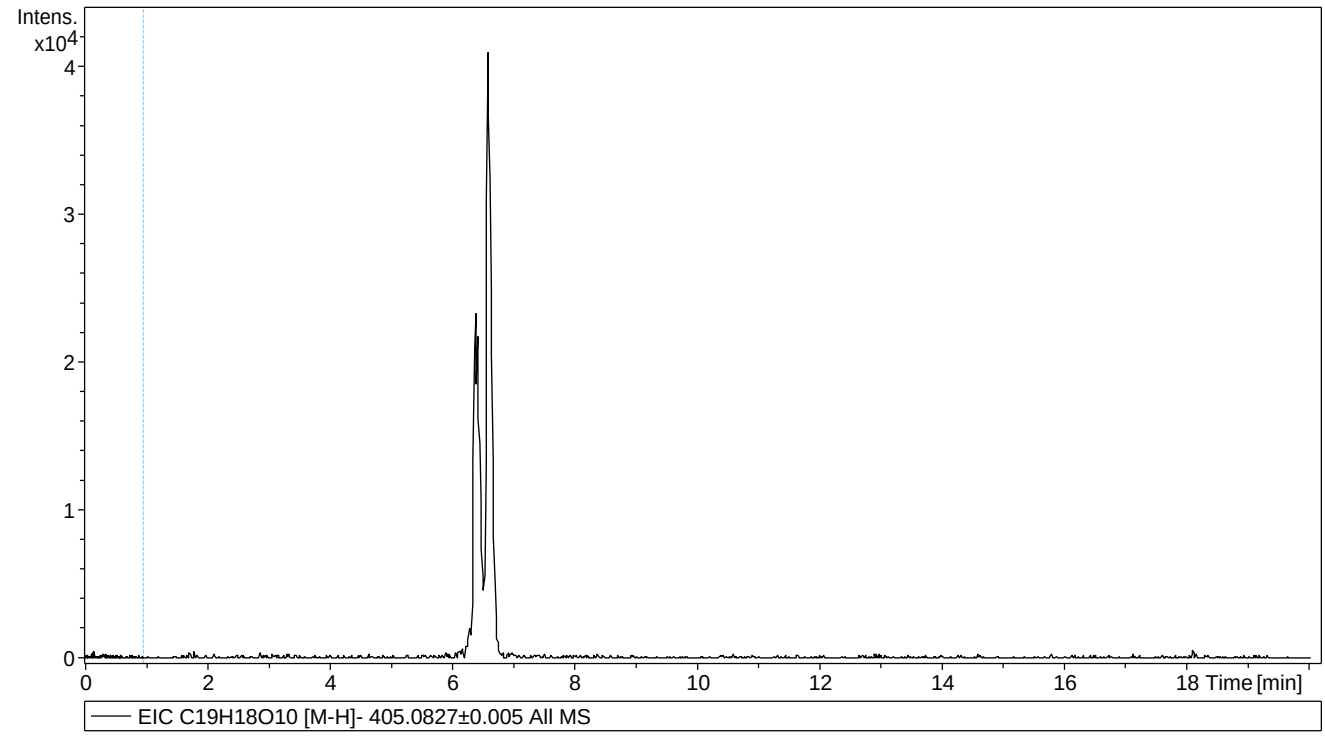
Operator BDAL@DE

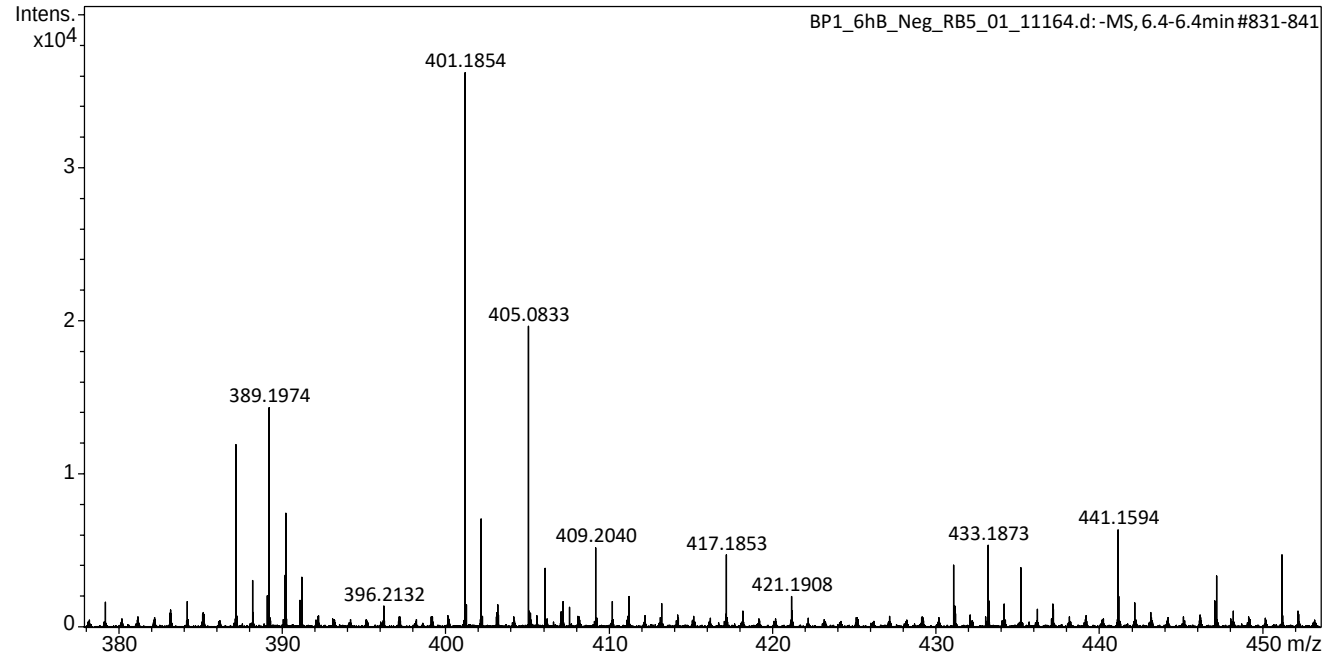
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

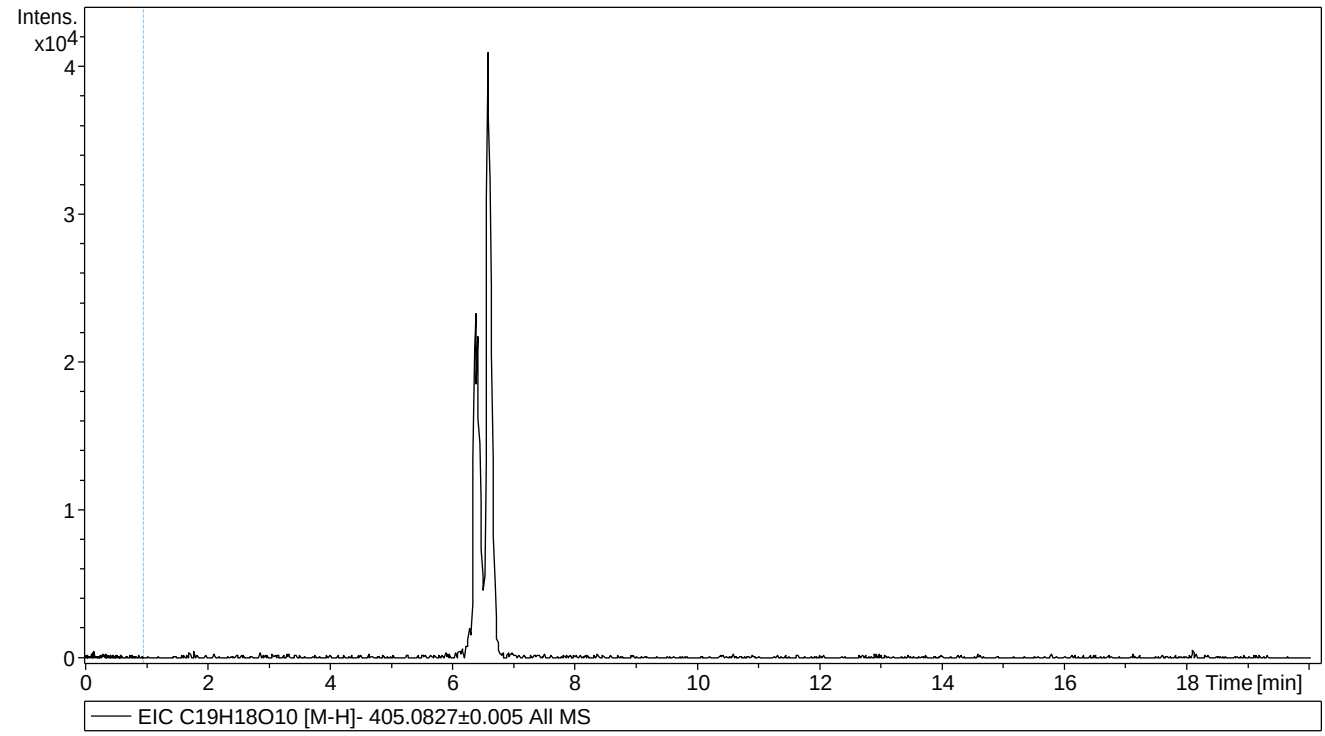
Operator BDAL@DE

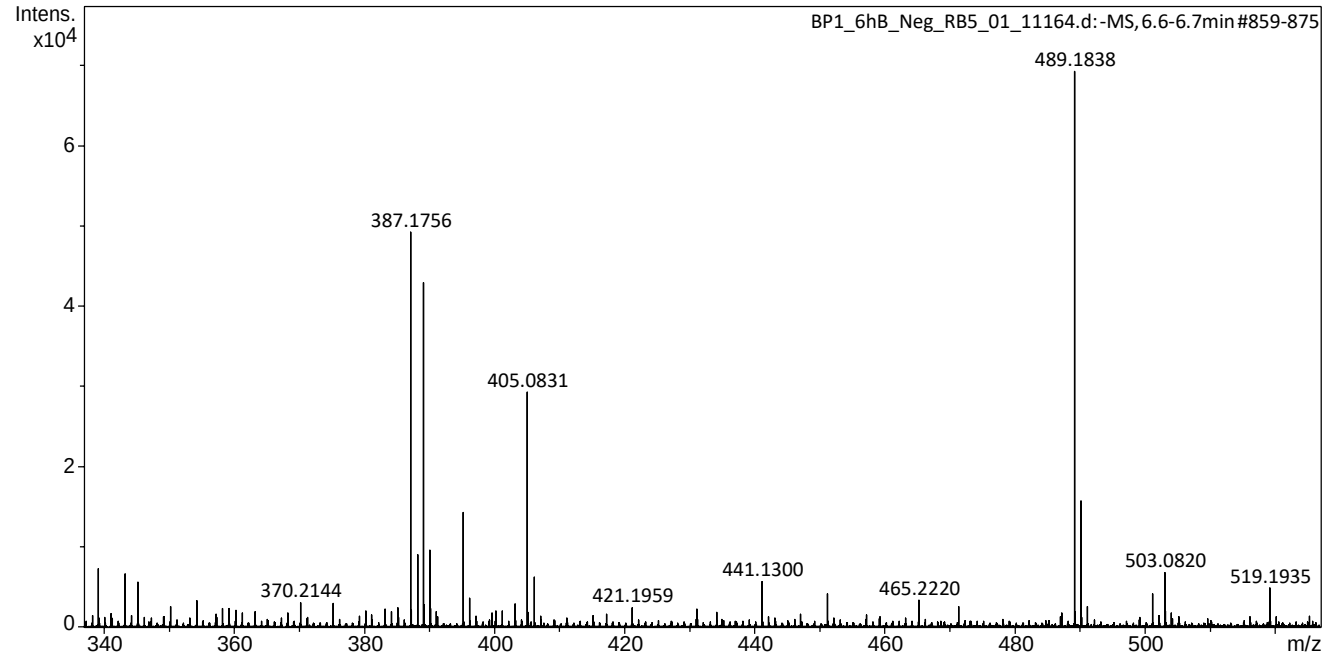
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

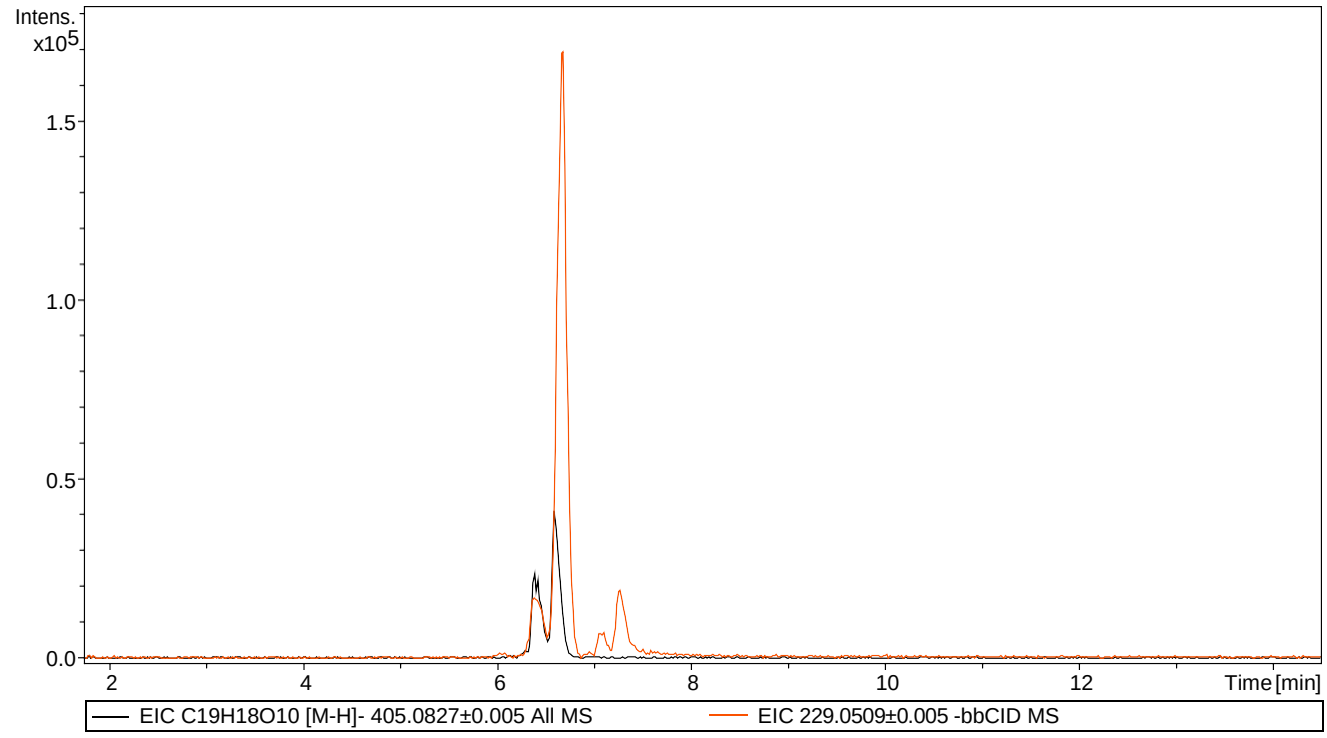
Operator BDAL@DE

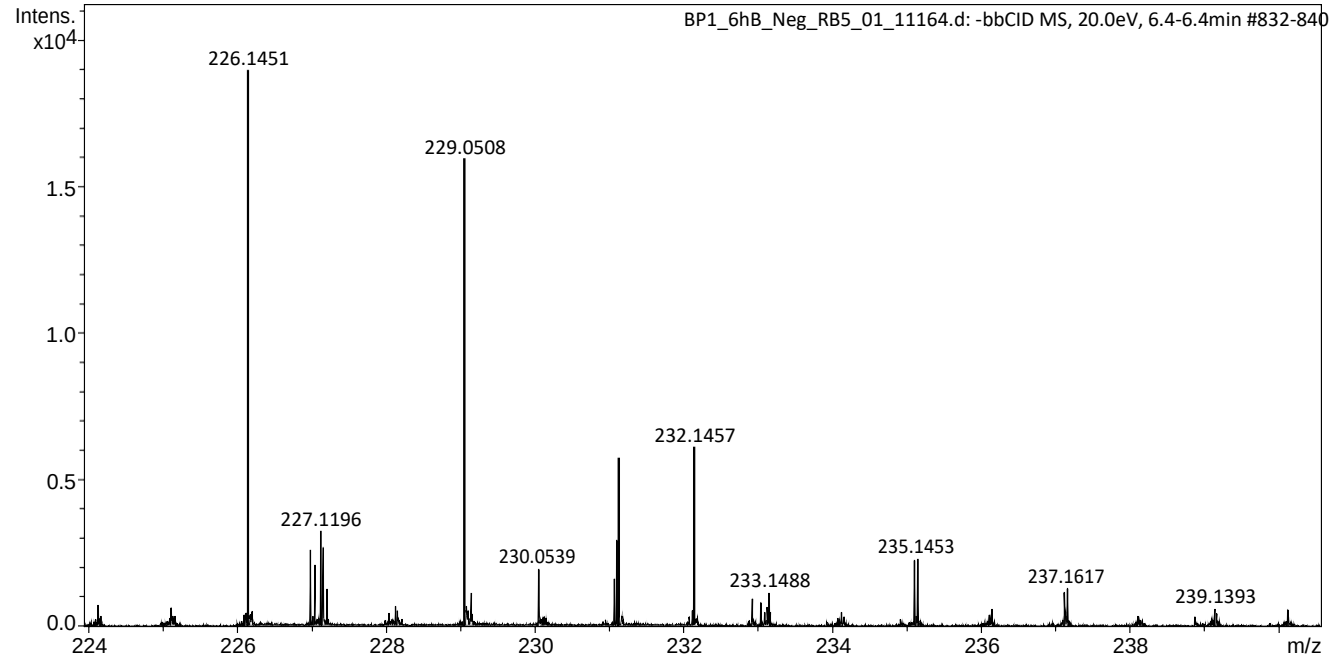
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

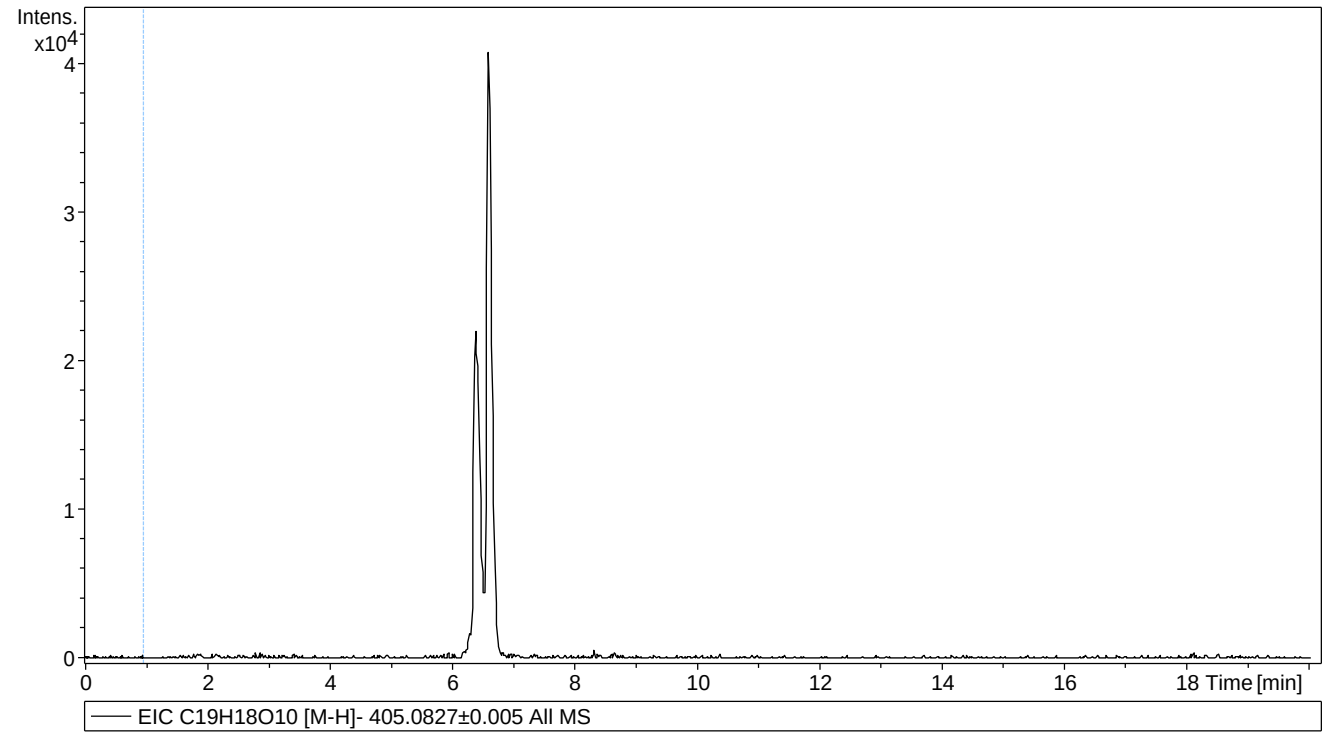
Operator BDAL@DE

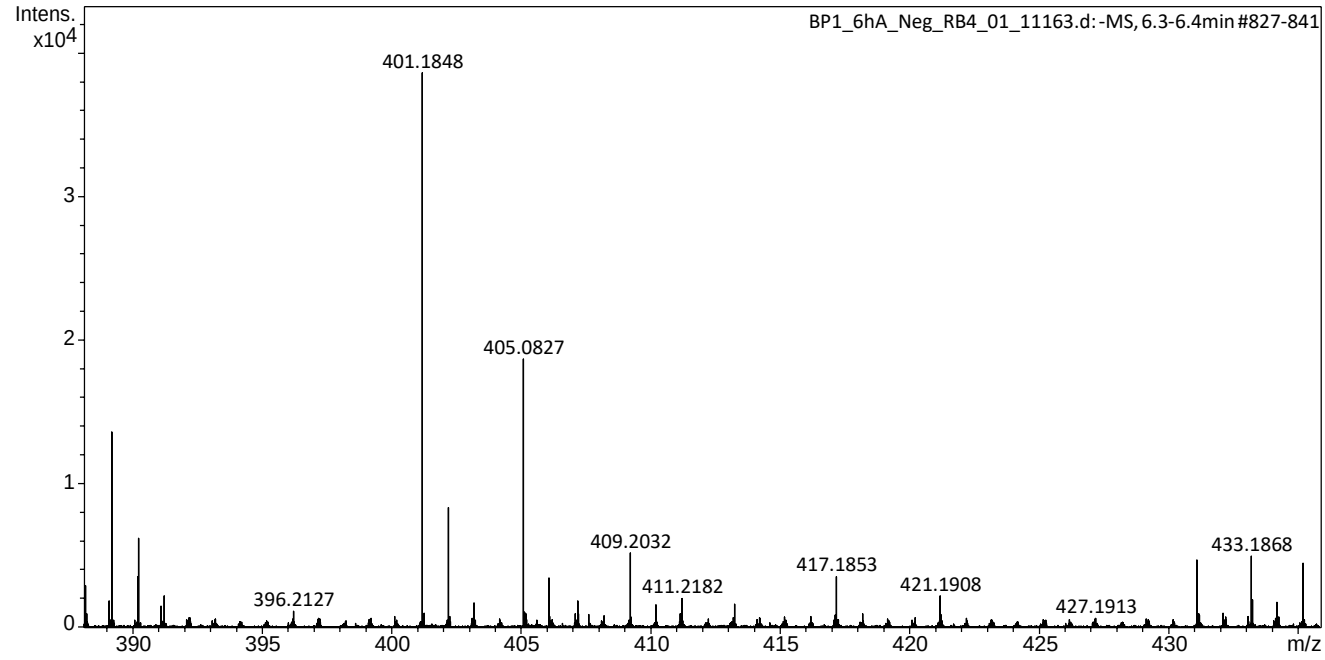
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

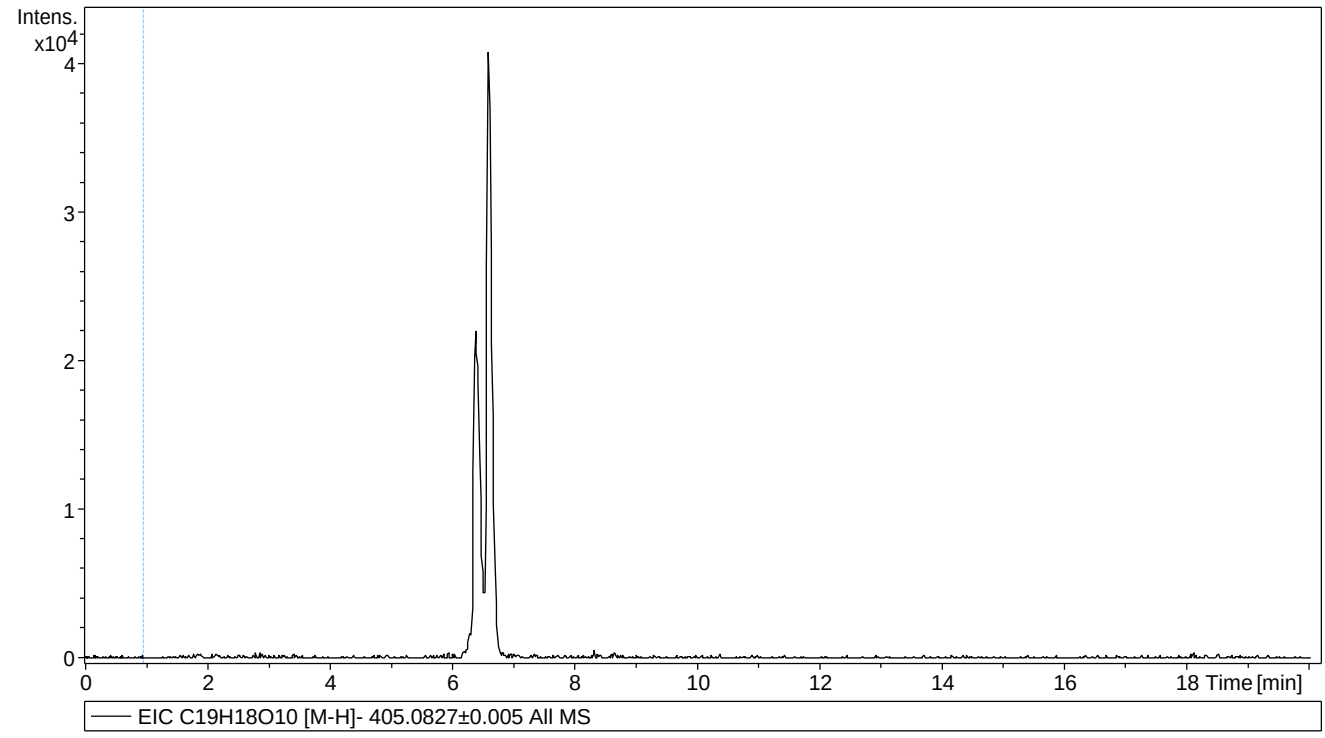
Operator BDAL@DE

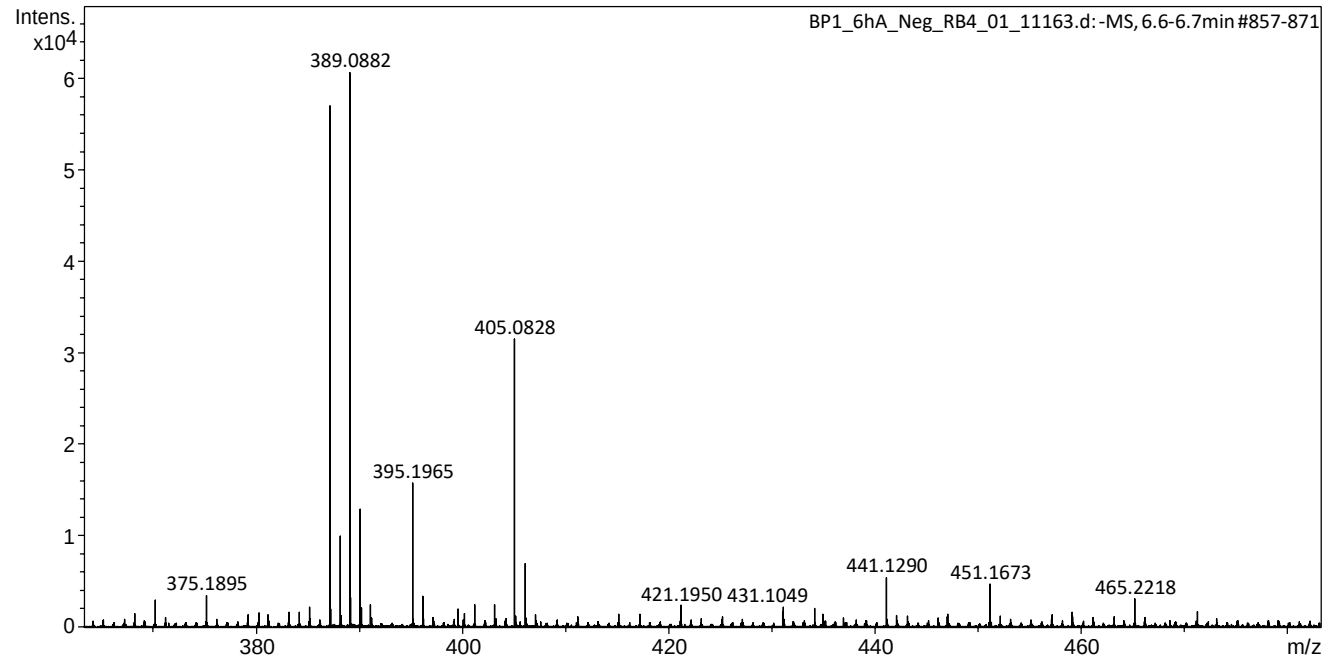
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hA_Neg_RB4_01_11163.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

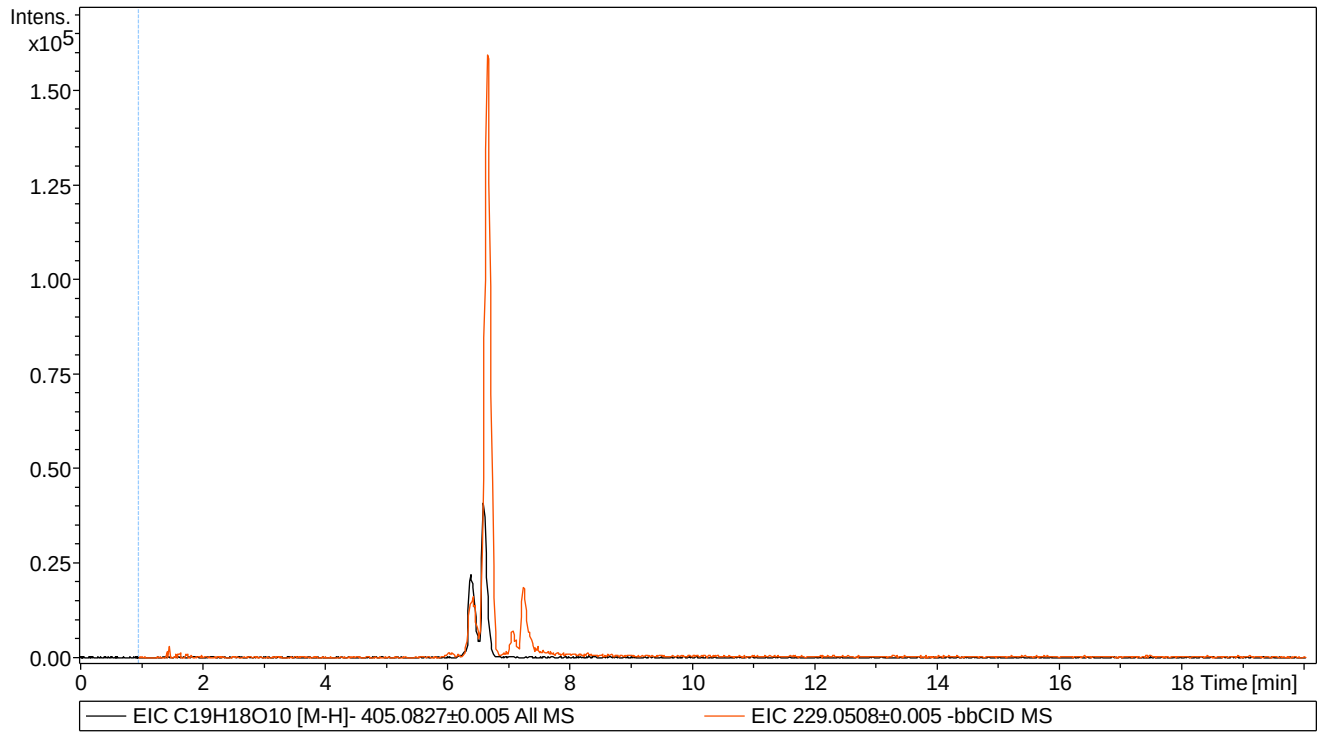
Operator BDAL@DE

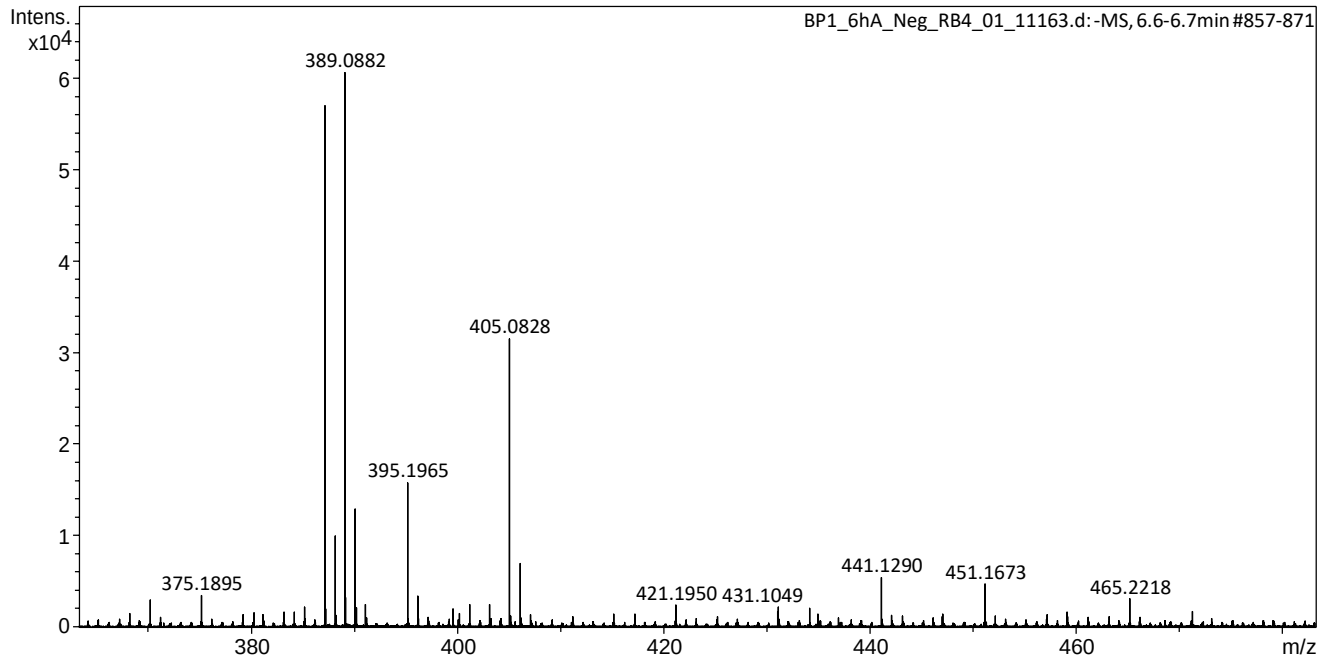
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

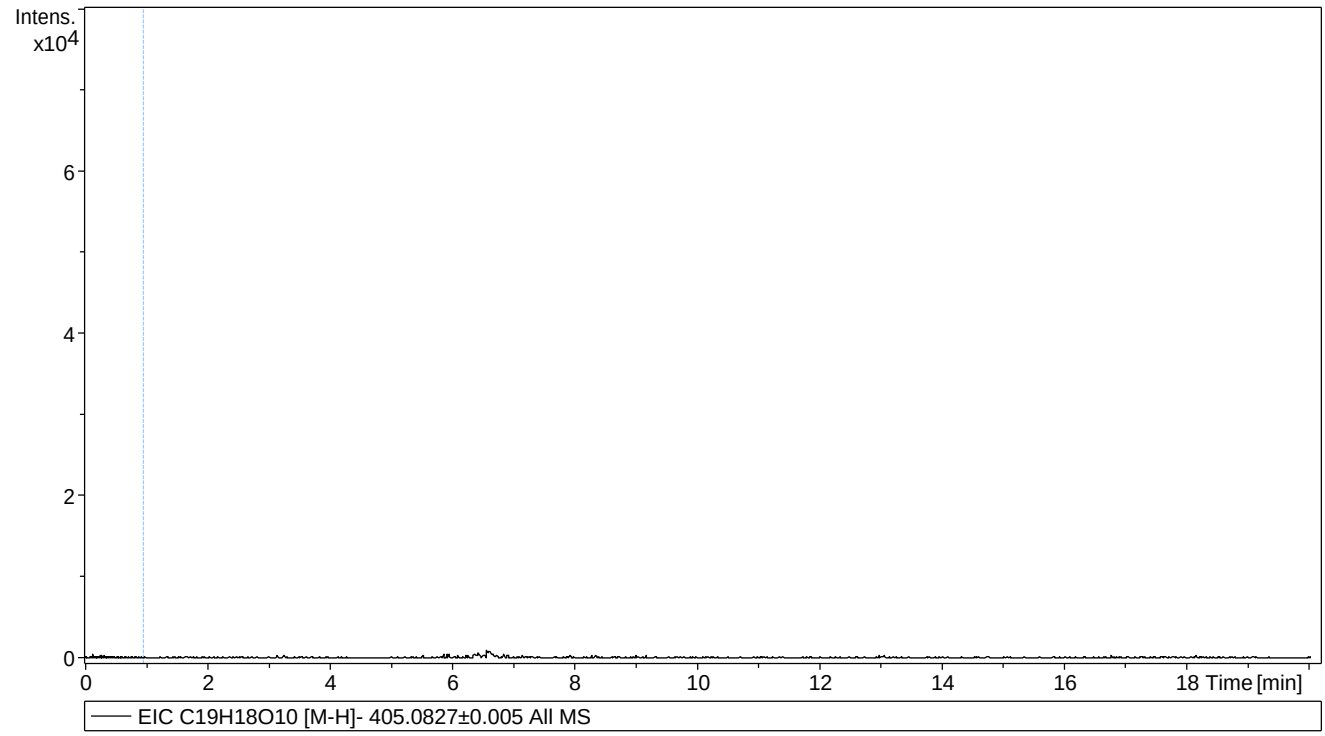
Operator BDAL@DE

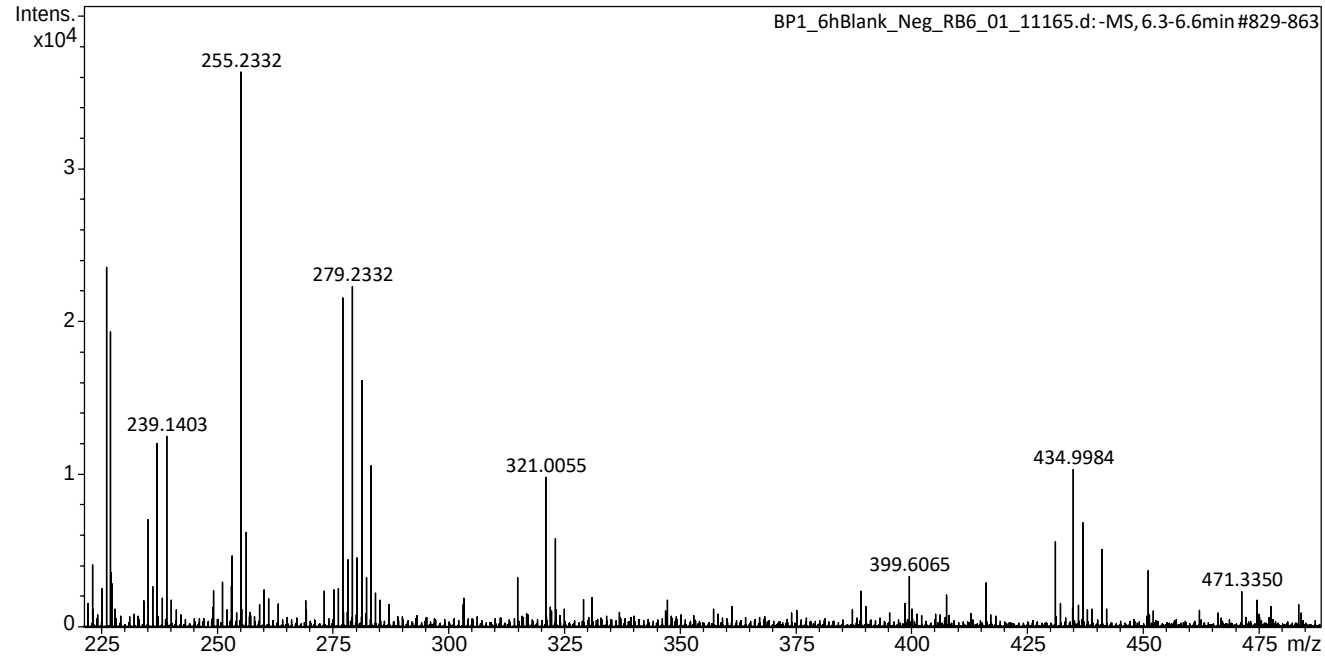
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

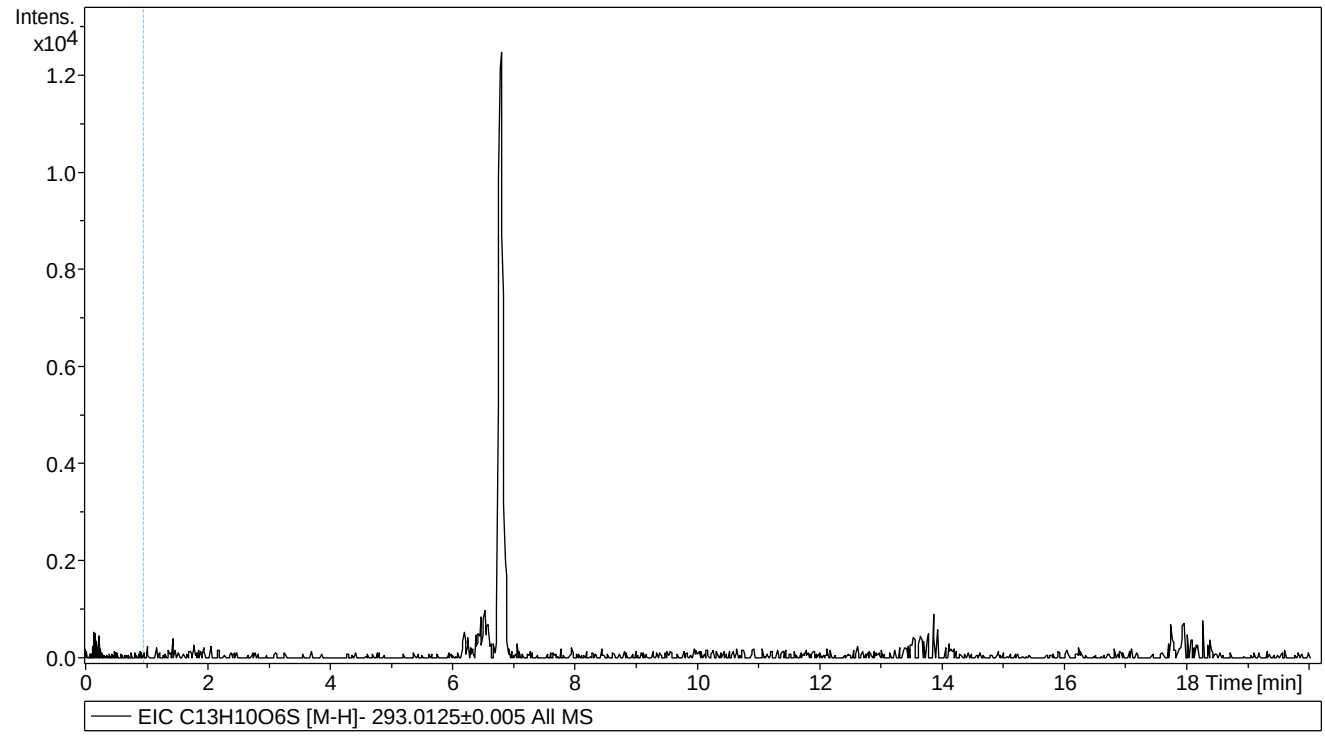
Operator BDAL@DE

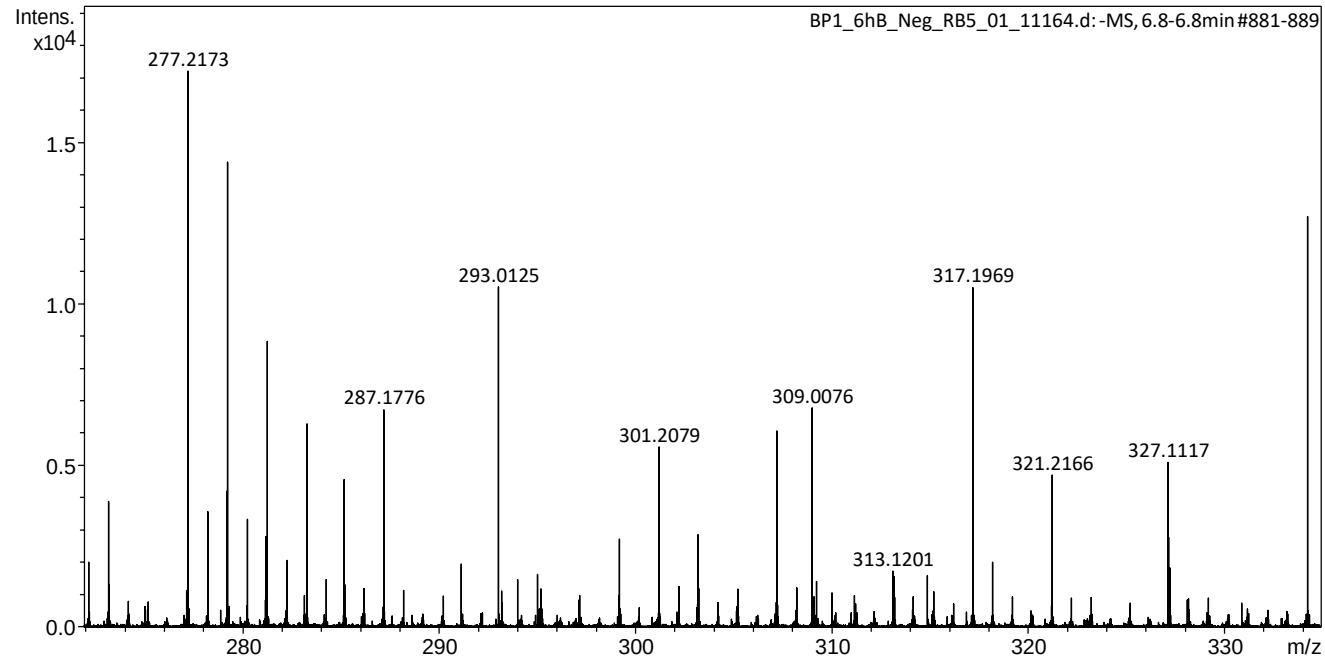
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hB_Neg_RB5_01_11164.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

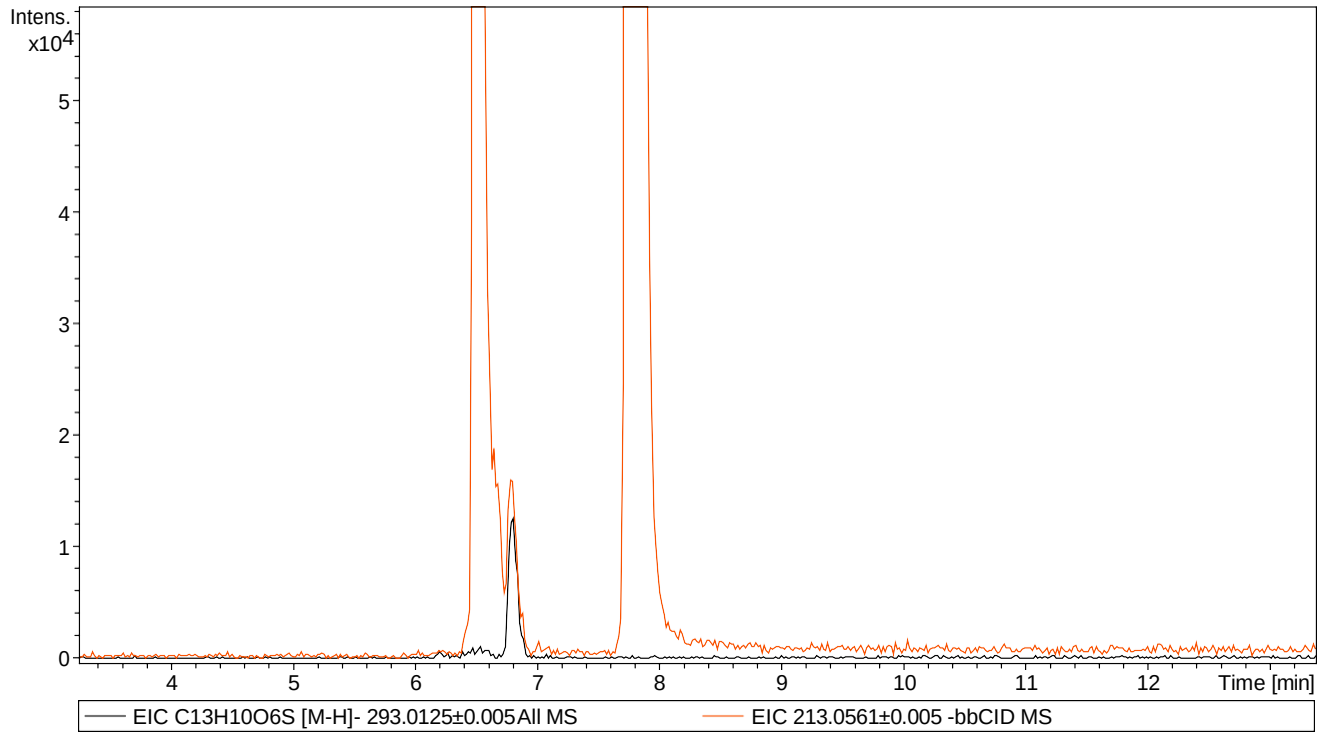
Operator BDAL@DE

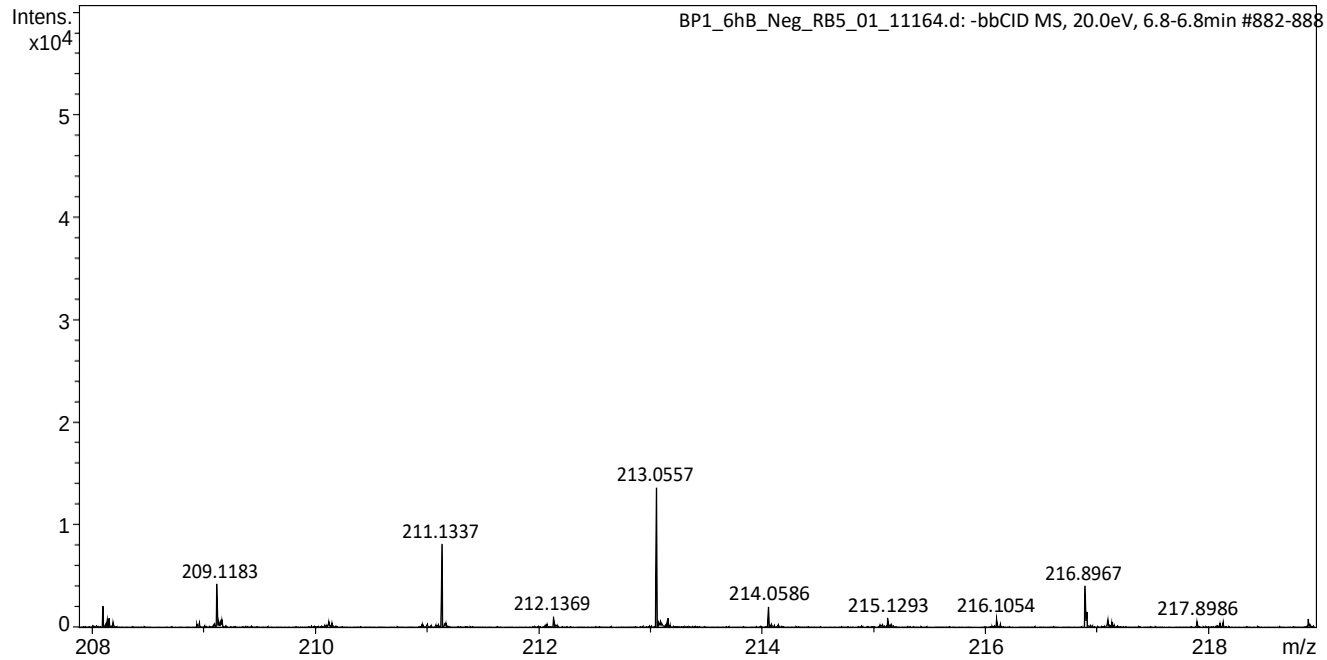
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

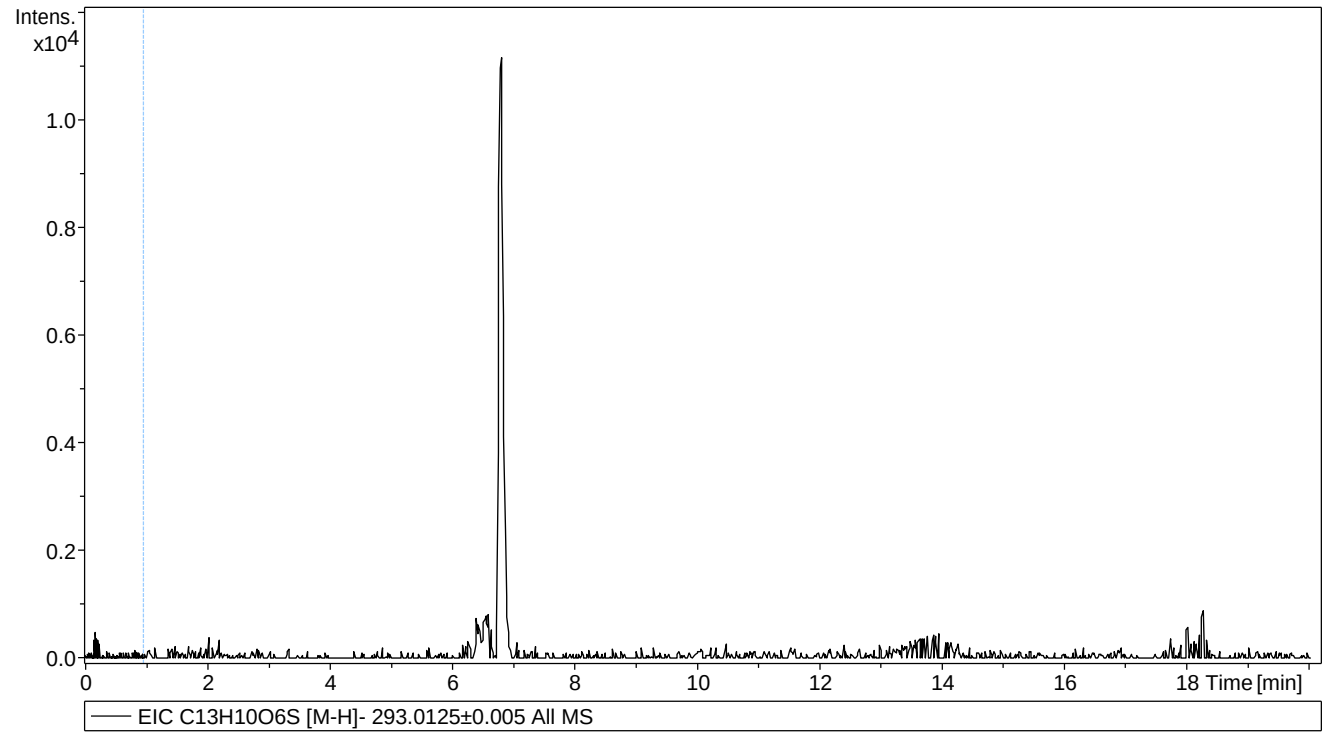
Operator BDAL@DE

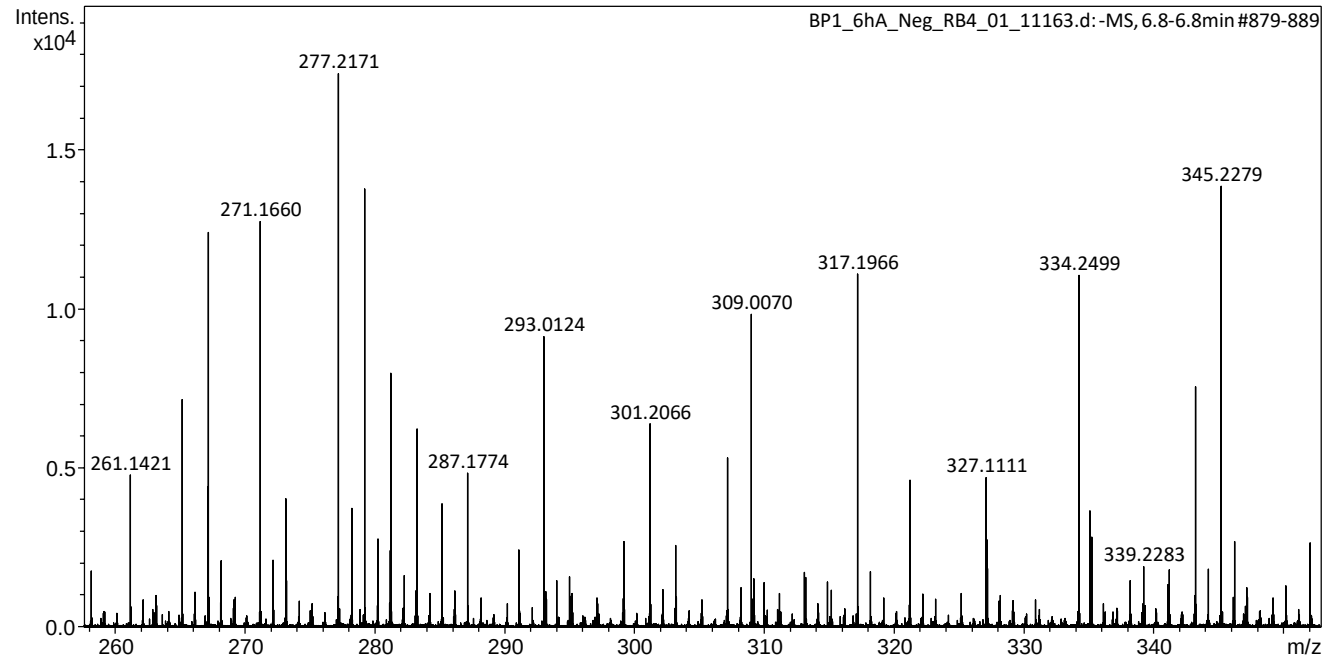
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

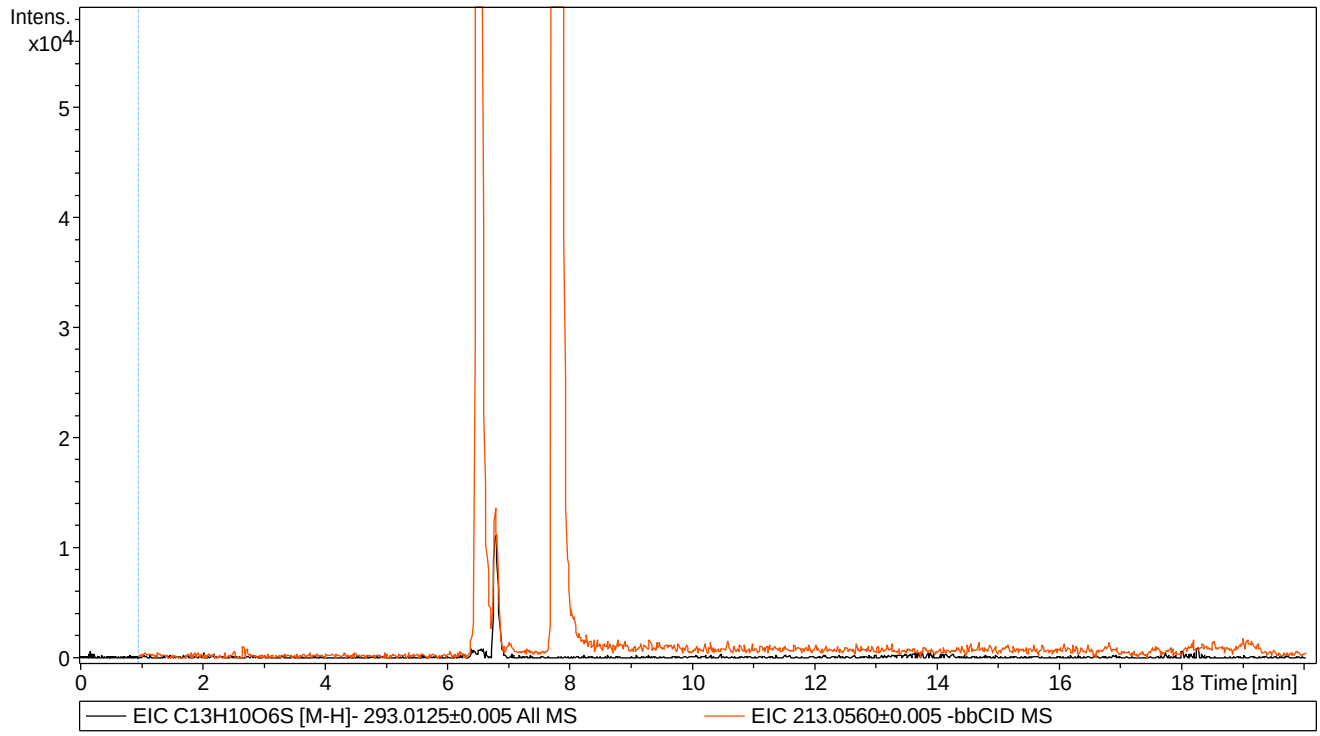
Operator BDAL@DE

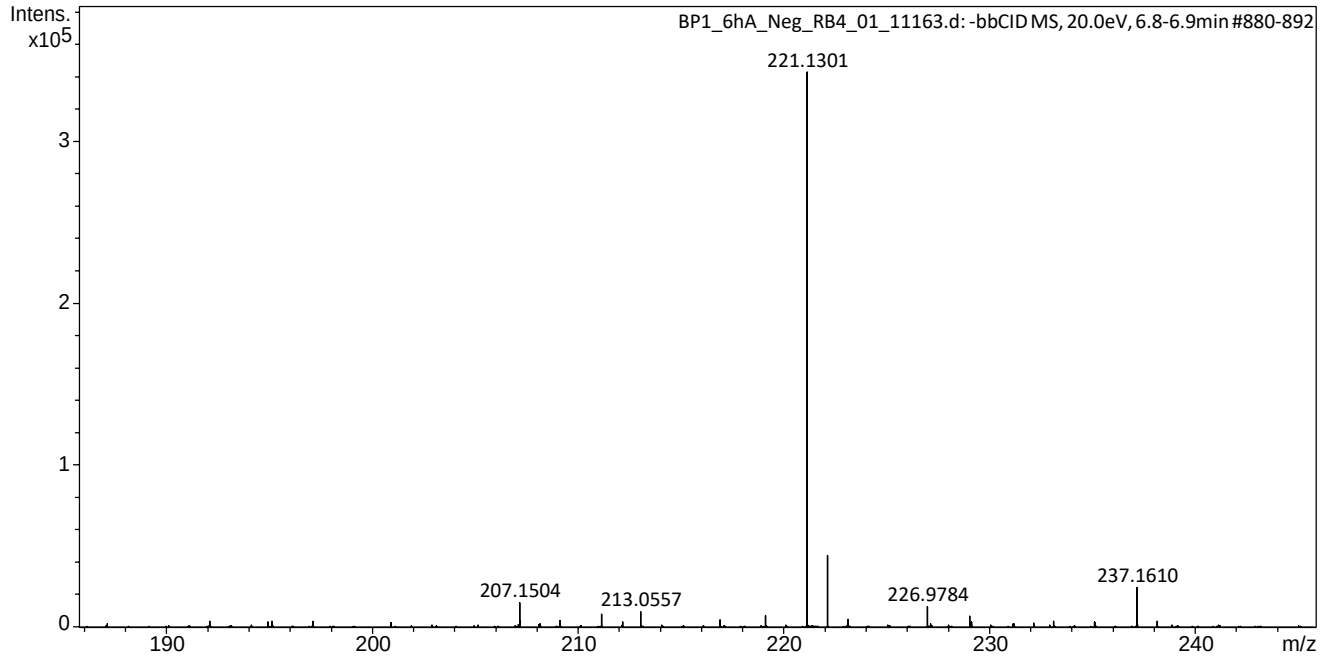
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hA_Neg_RB4_01_11163.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

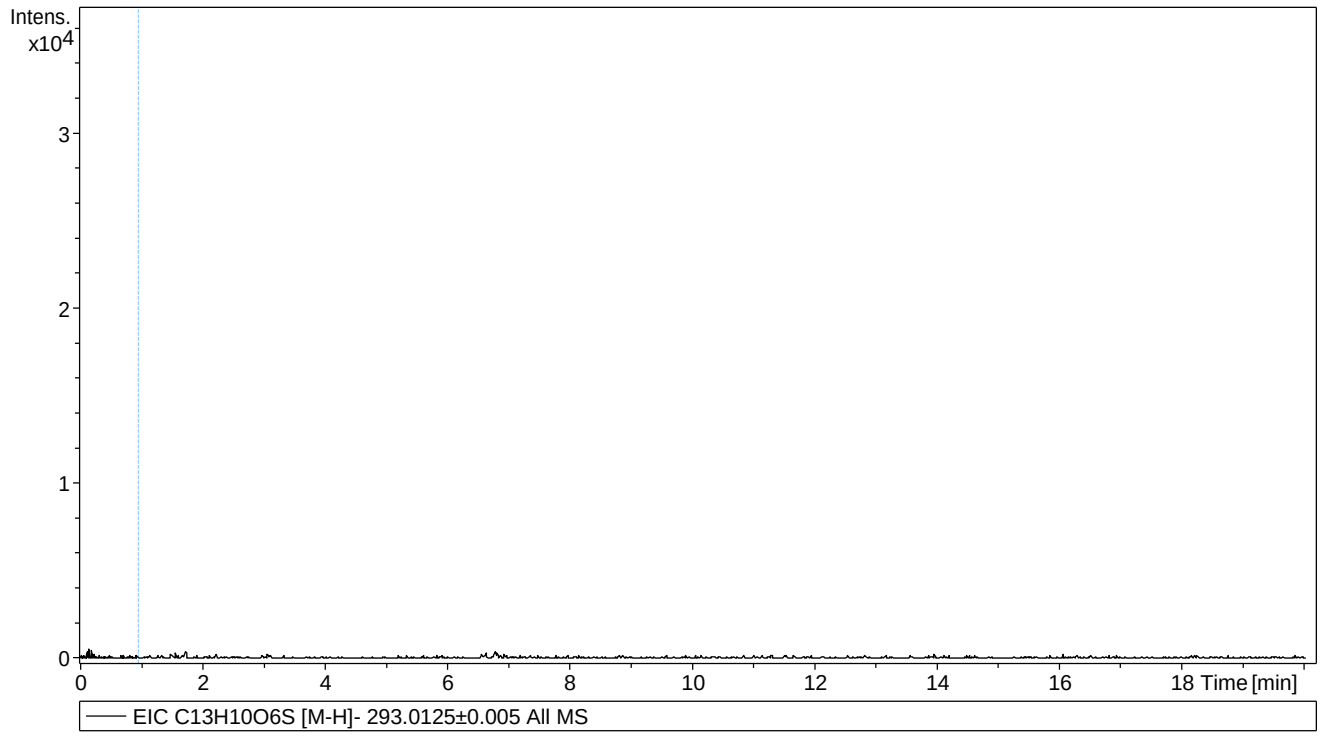
Operator BDAL@DE

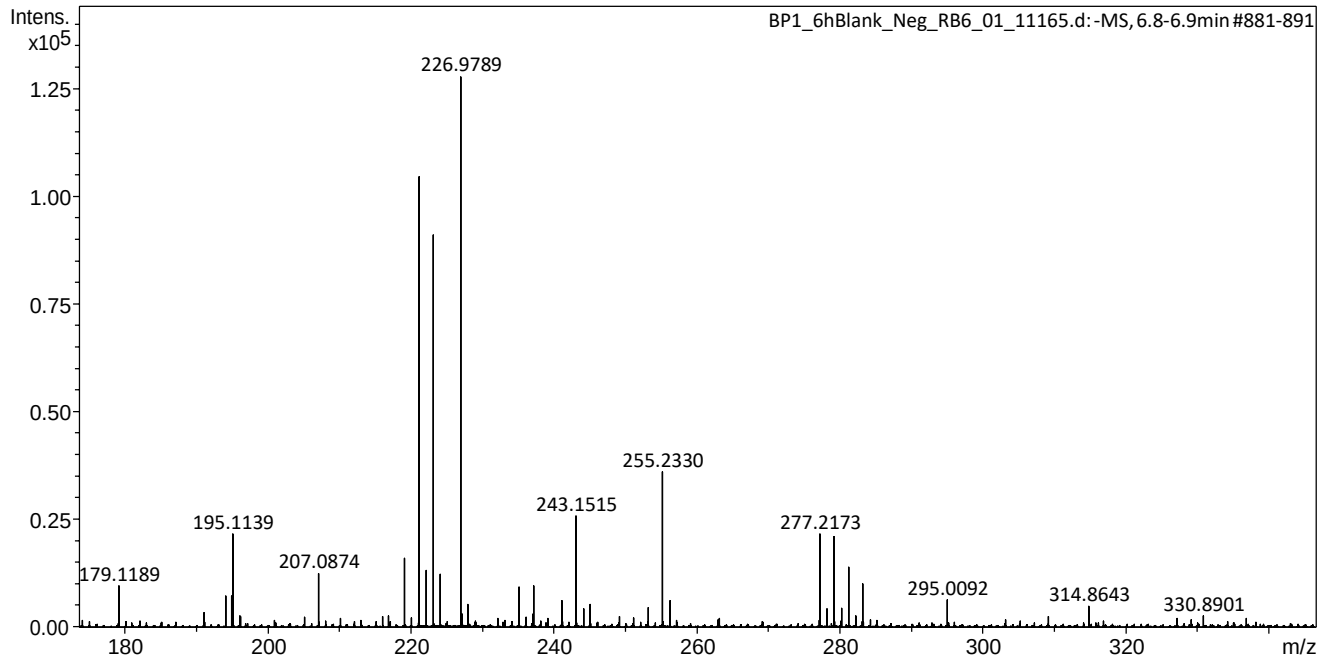
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

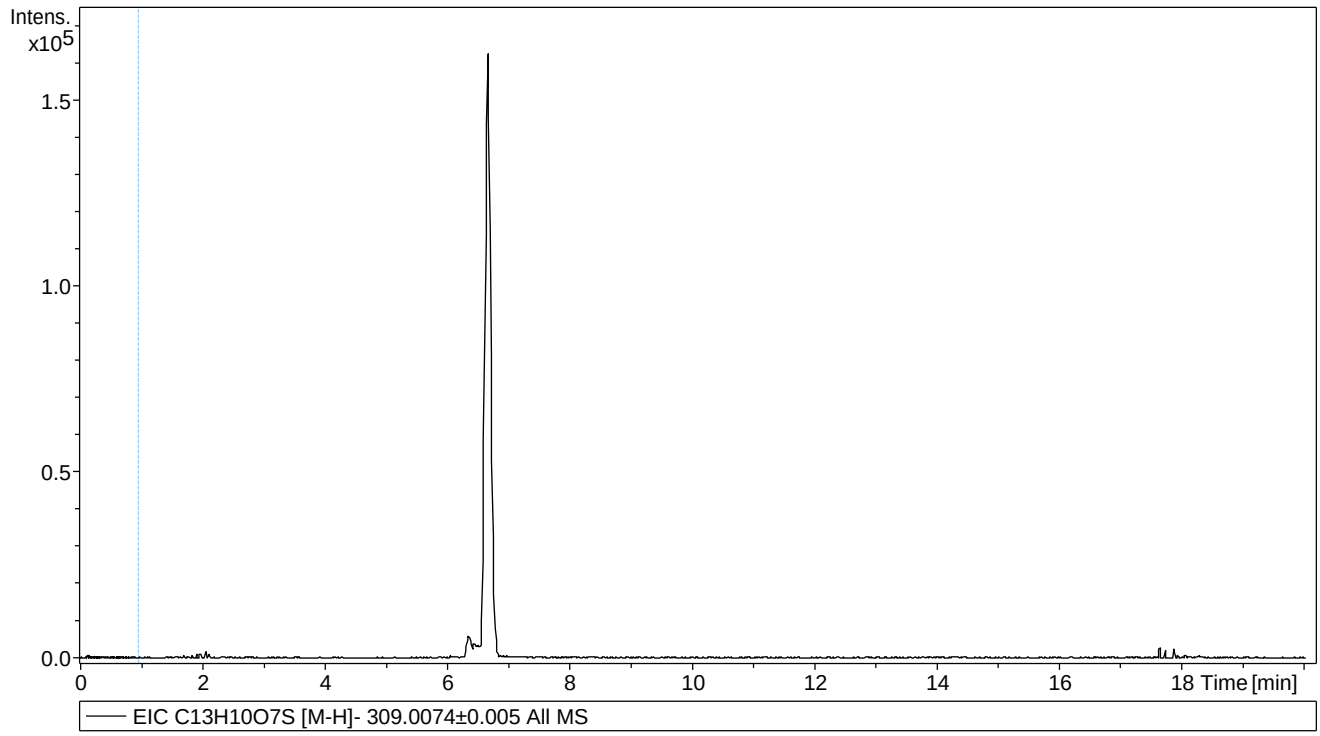
Operator BDAL@DE

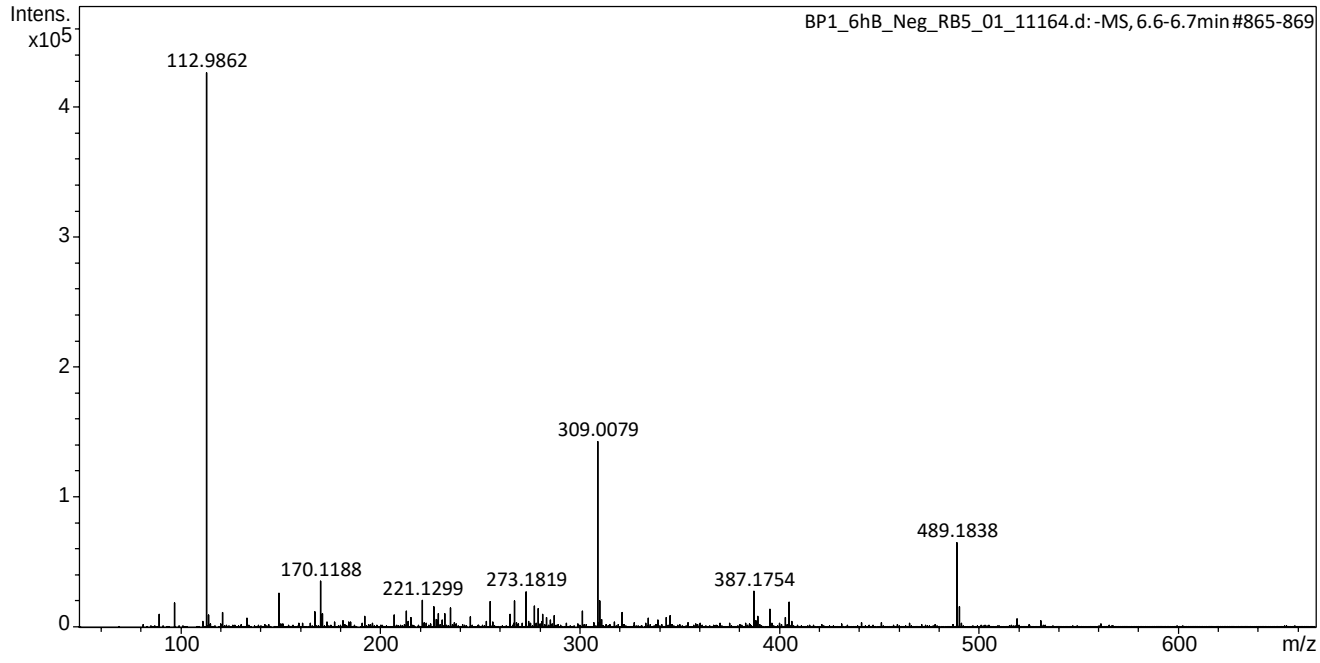
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hB_Neg_RB5_01_11164.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

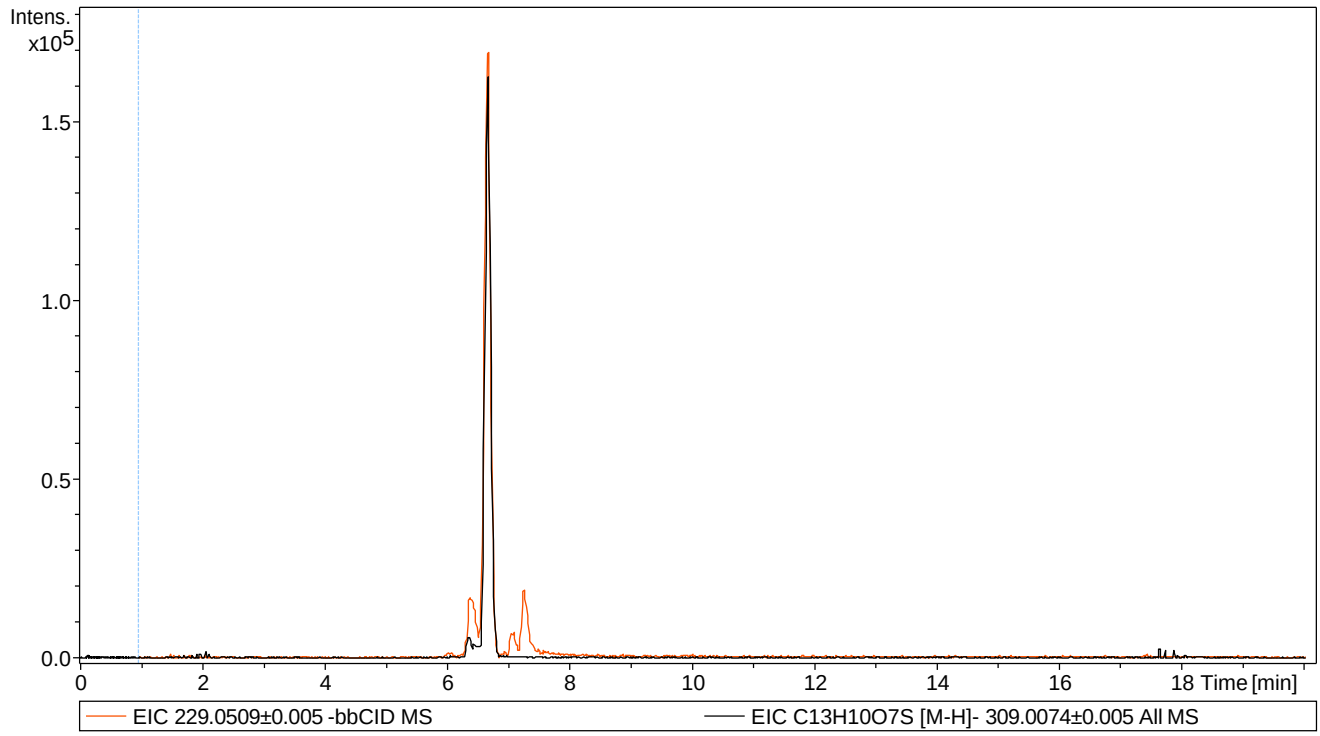
Operator BDAL@DE

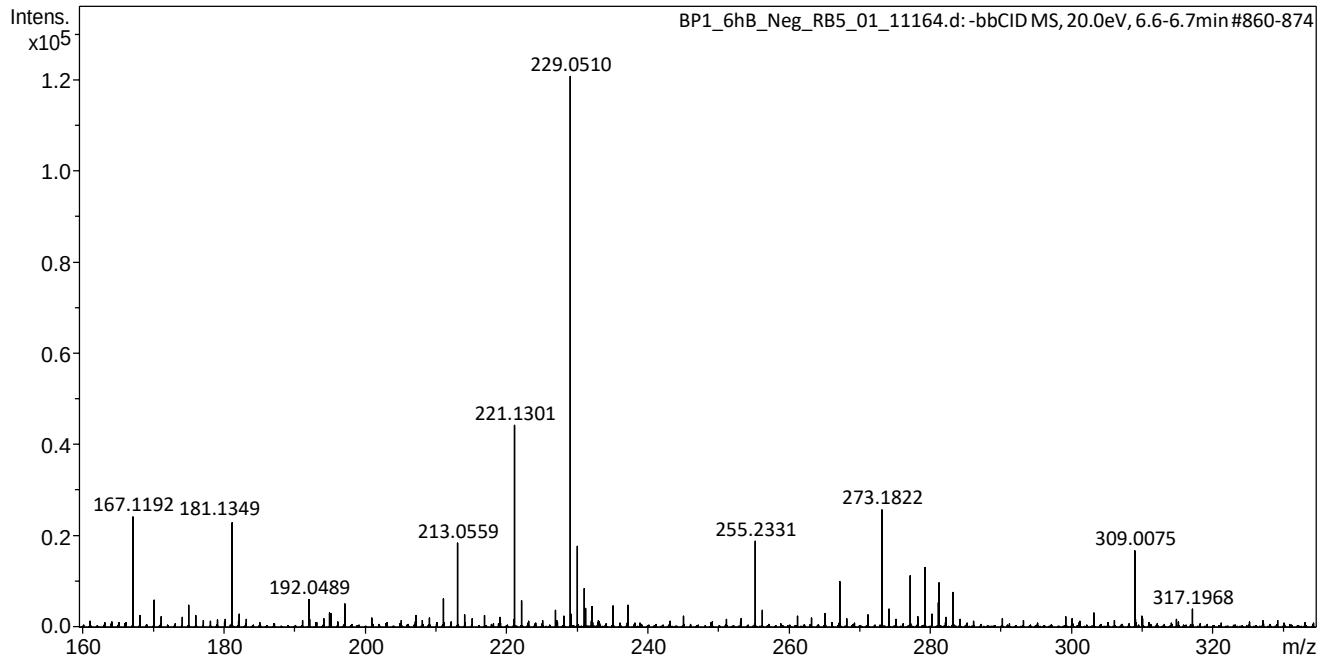
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

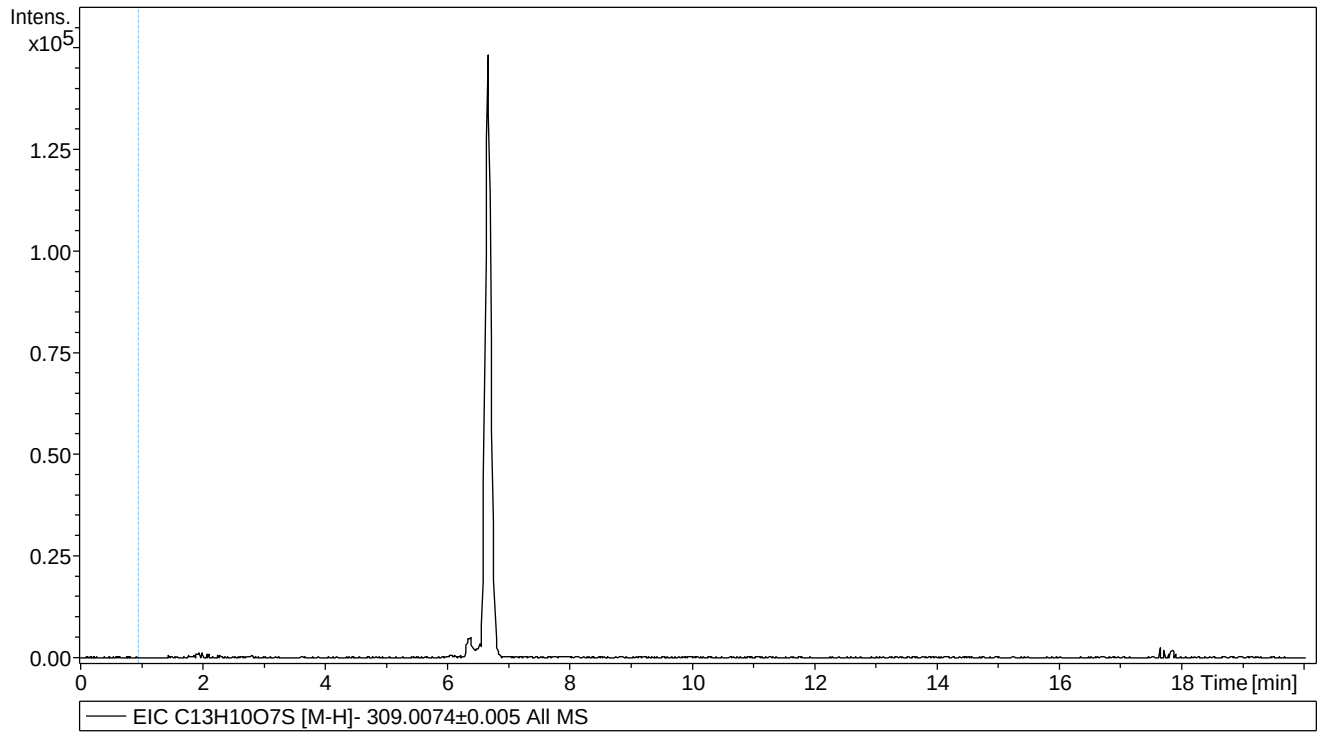
Operator BDAL@DE

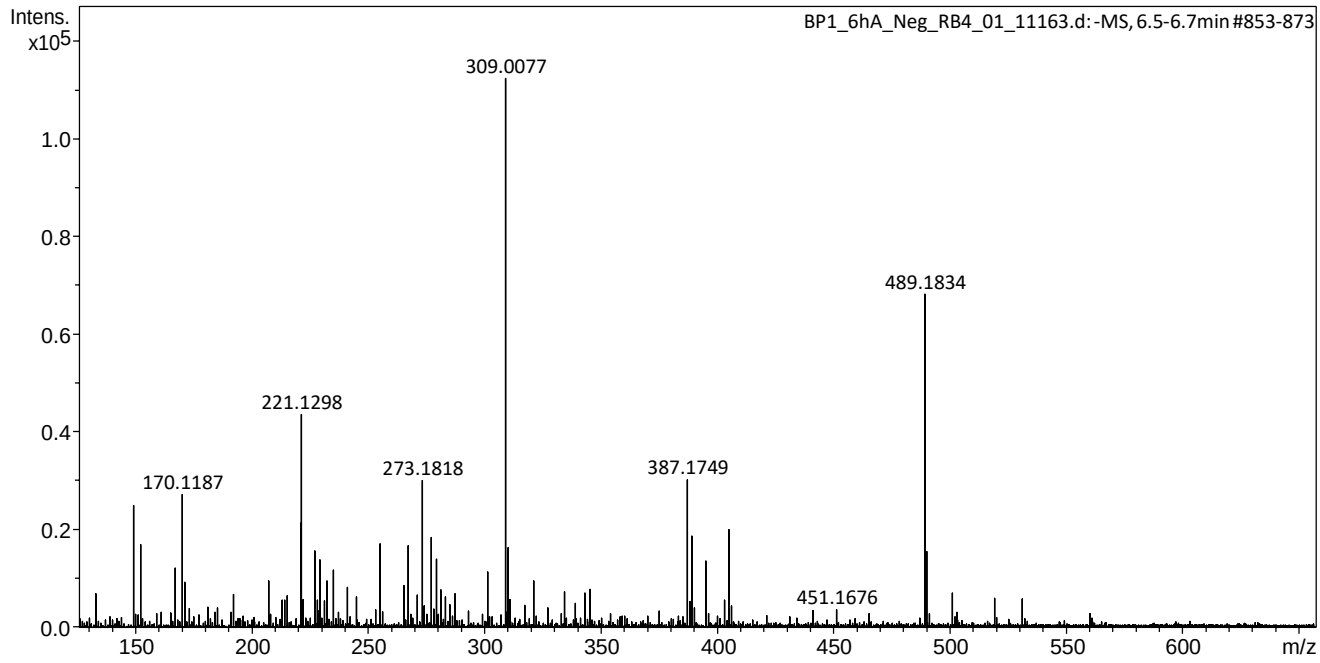
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

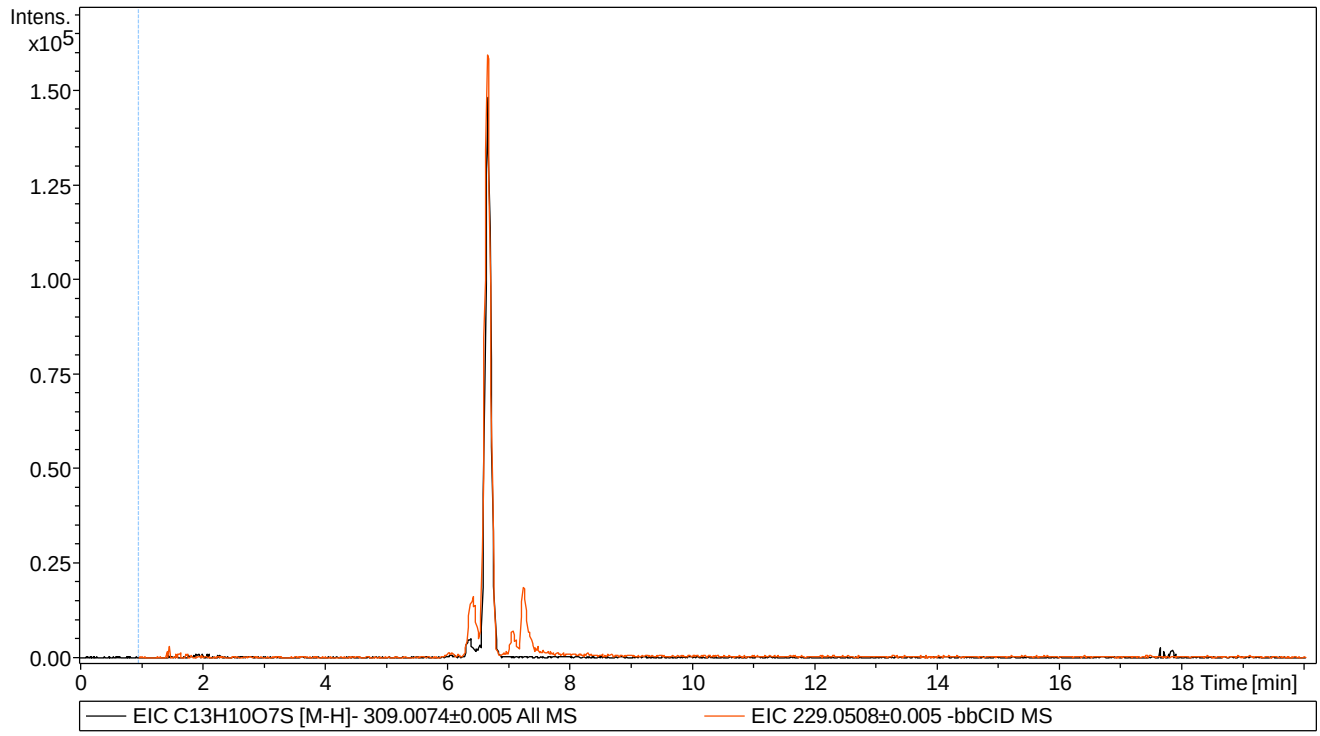
Operator BDAL@DE

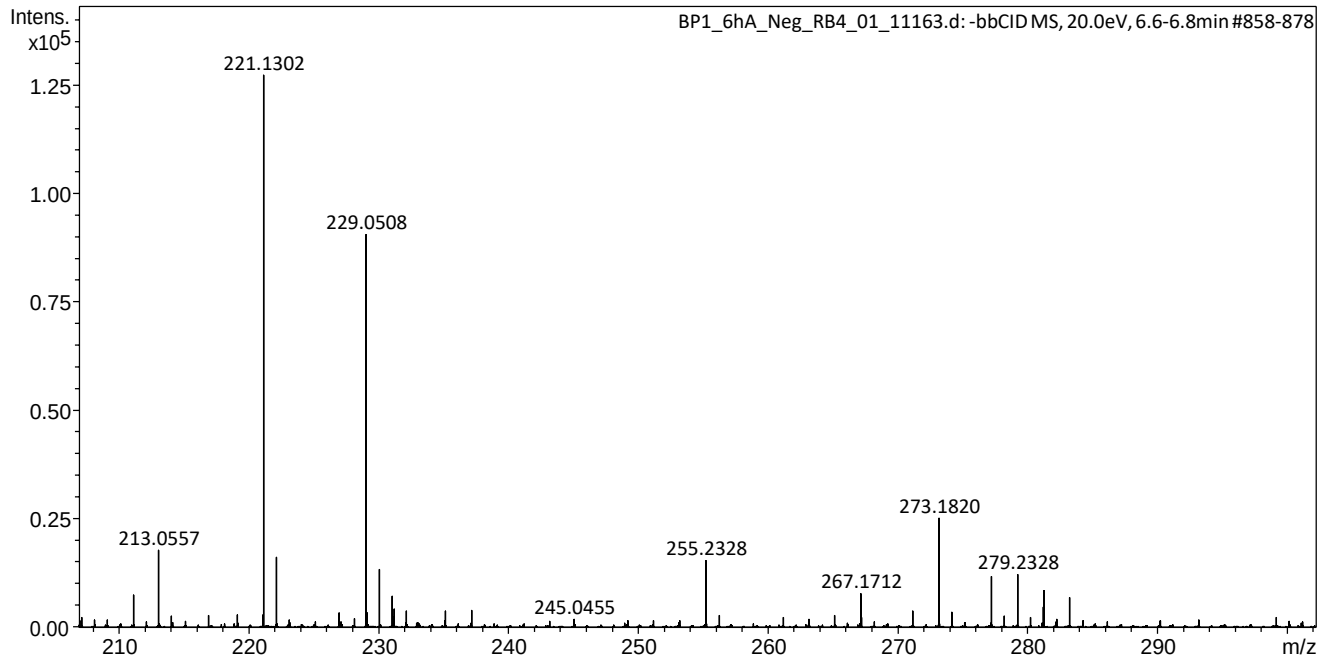
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hA_Neg_RB4_01_11163.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

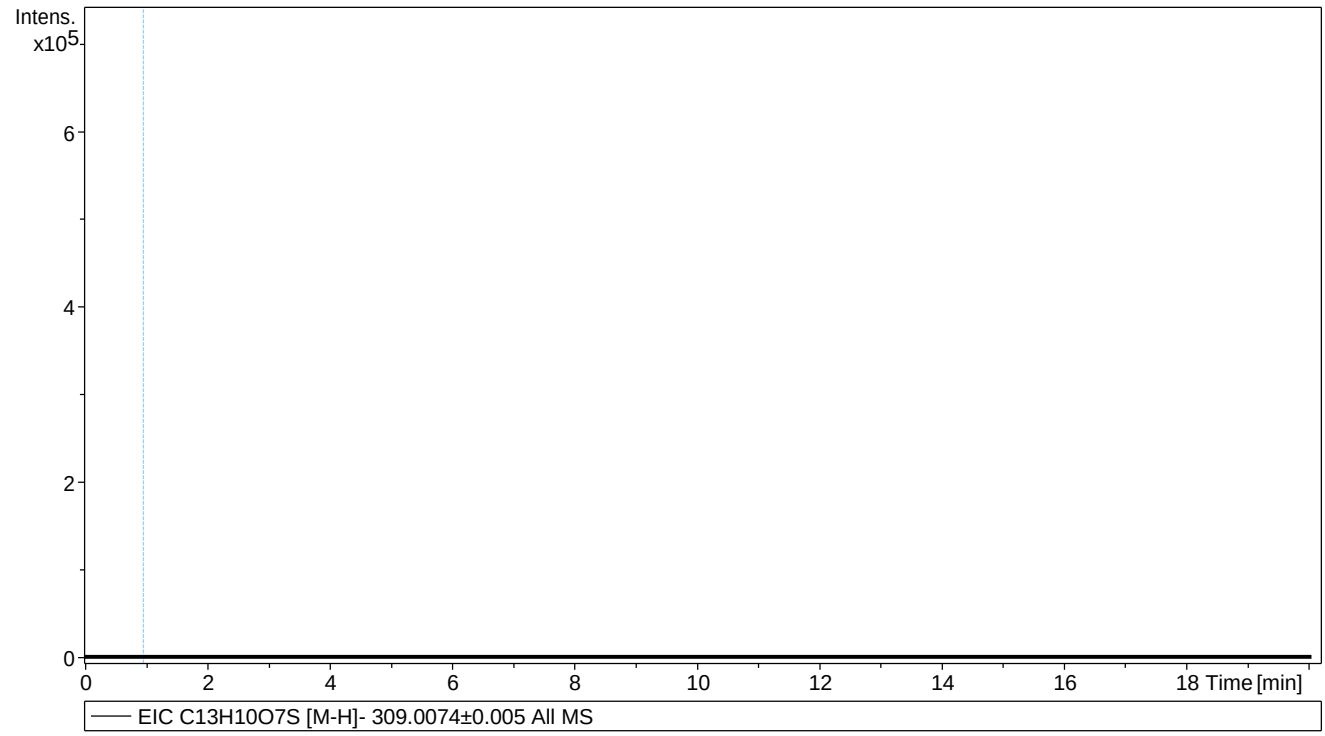
Operator BDAL@DE

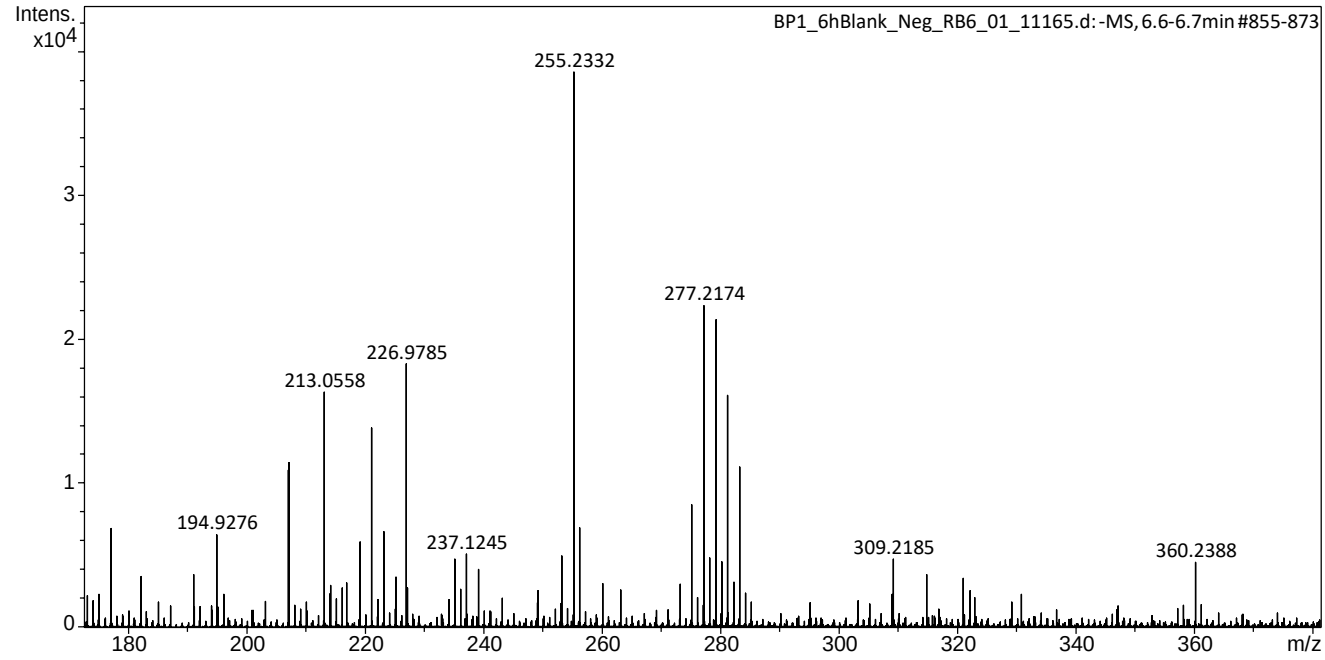
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

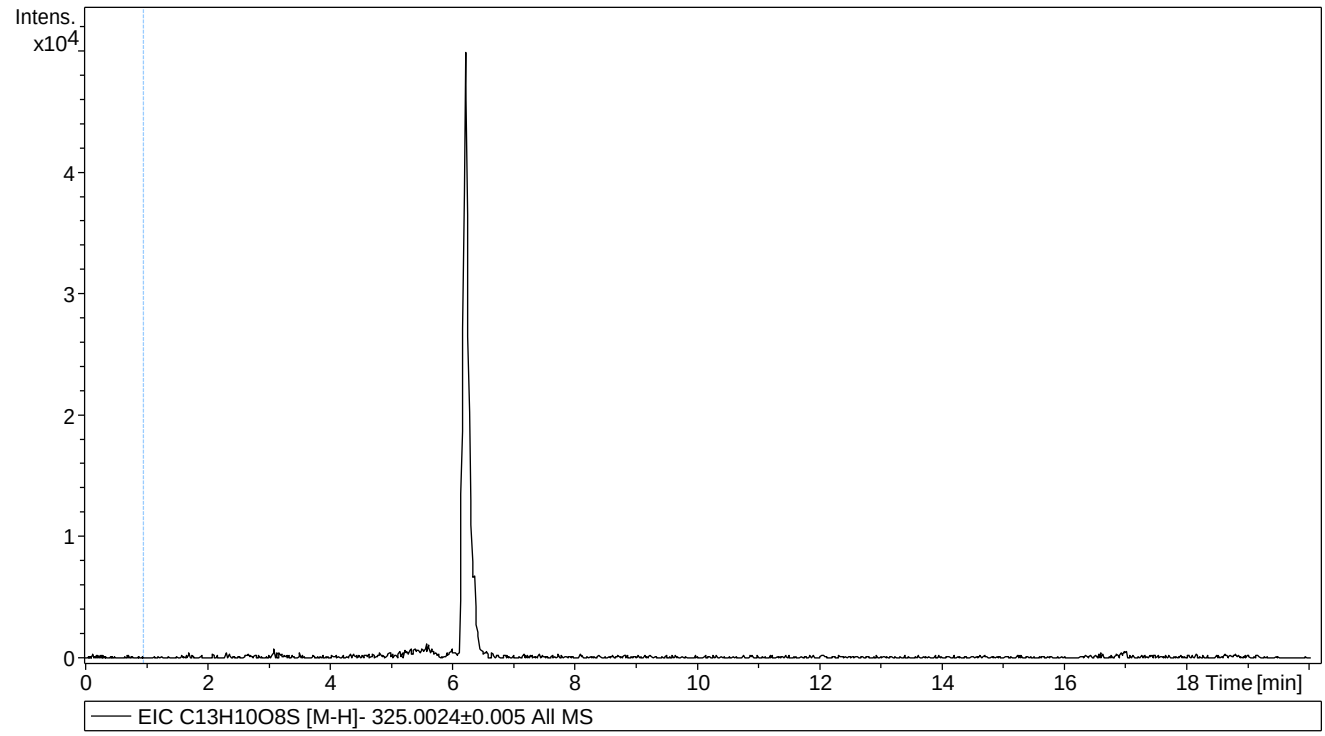
Operator BDAL@DE

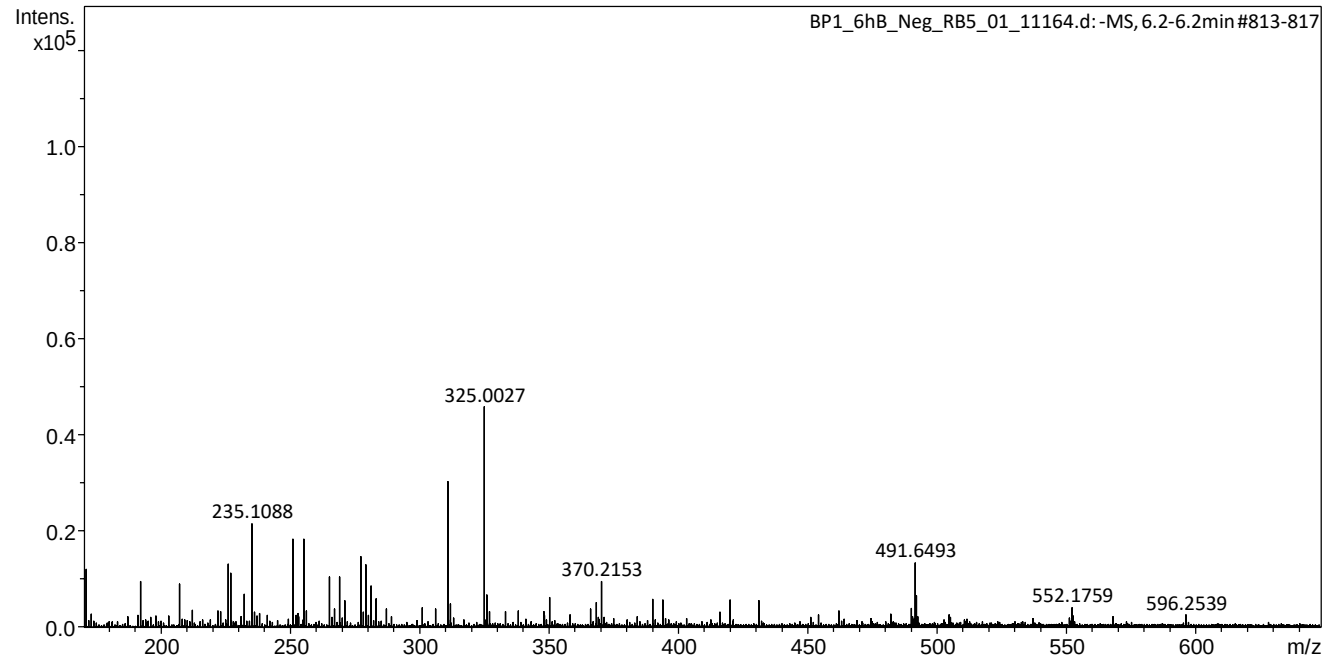
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hB_Neg_RB5_01_11164.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

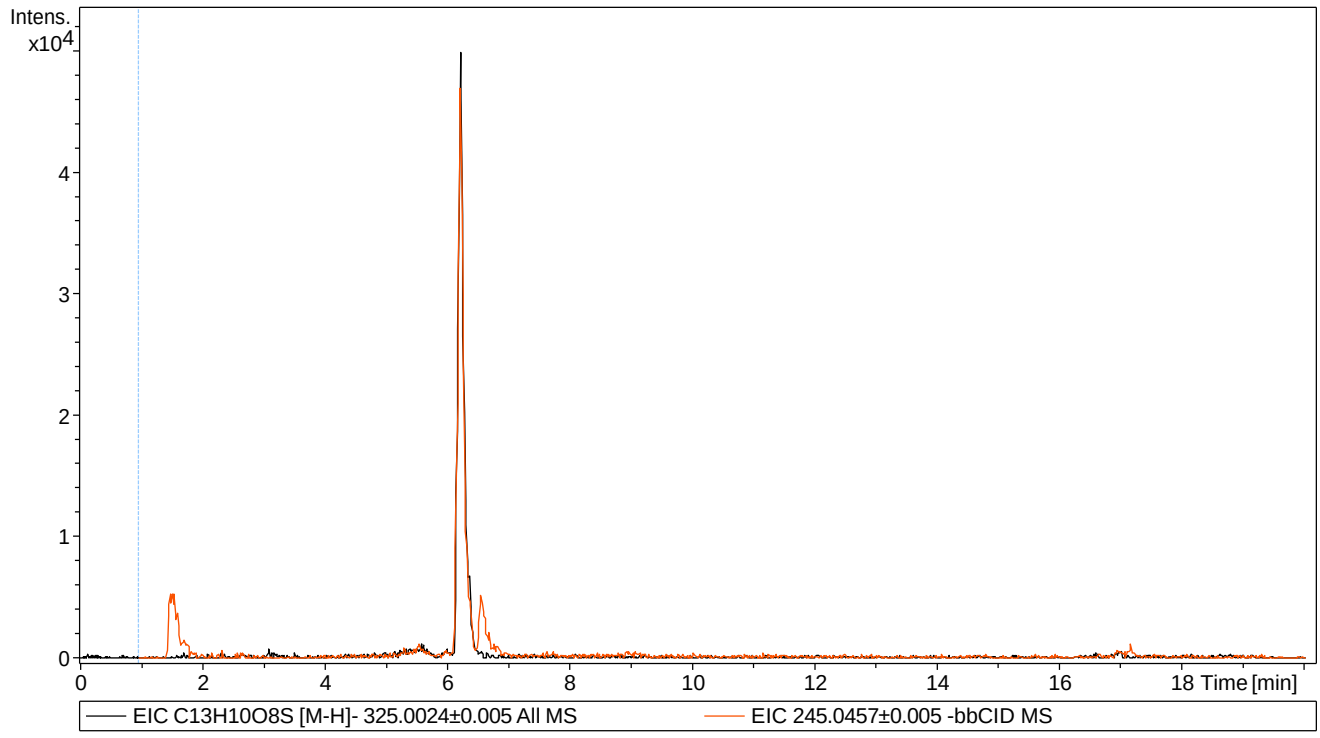
Operator BDAL@DE

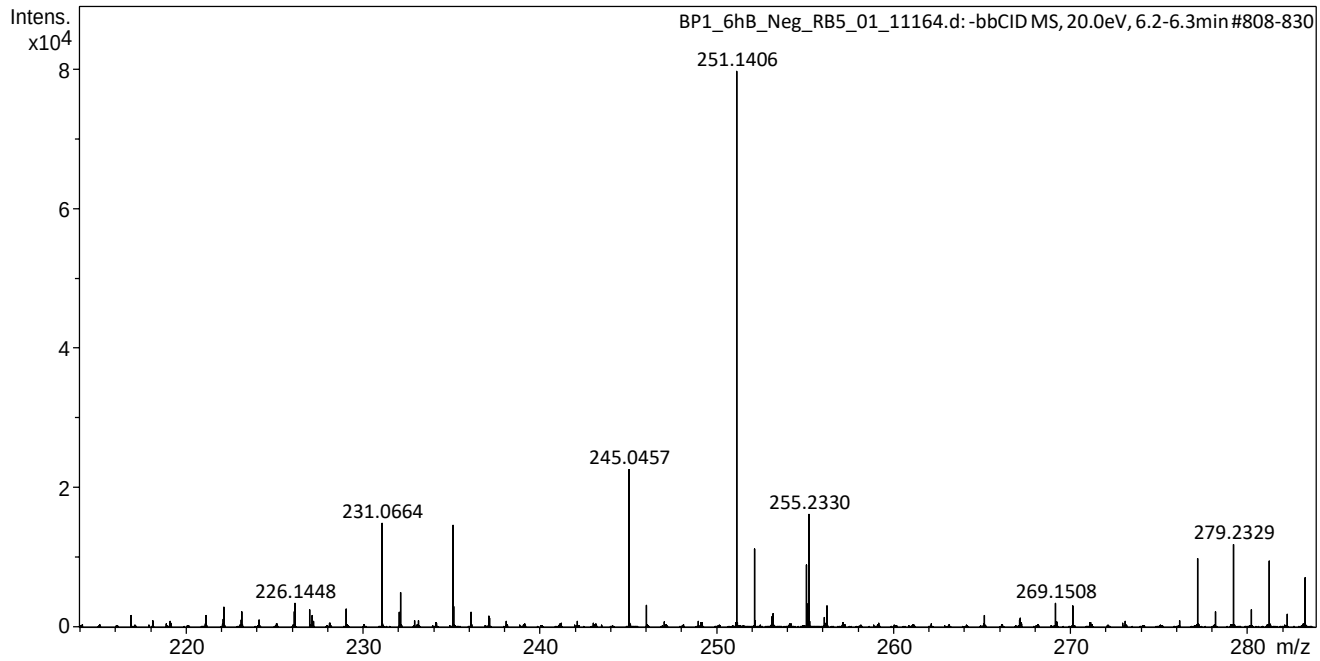
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

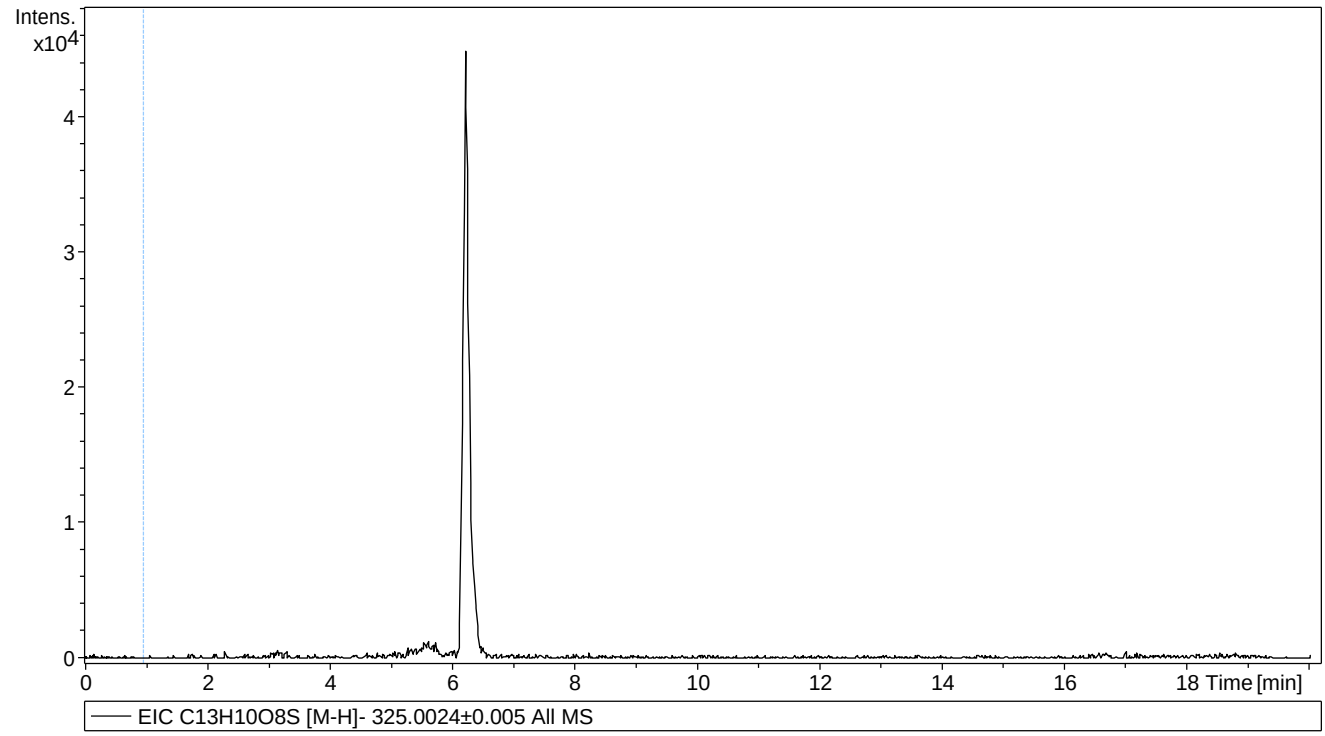
Operator BDAL@DE

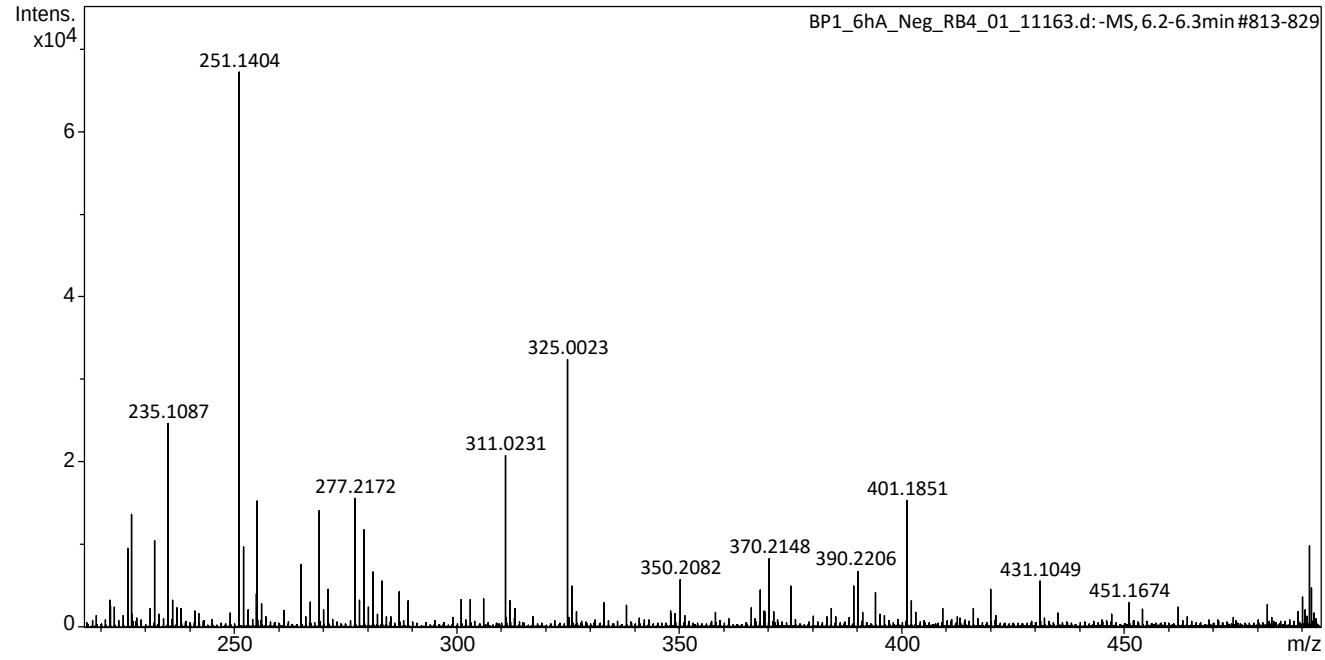
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

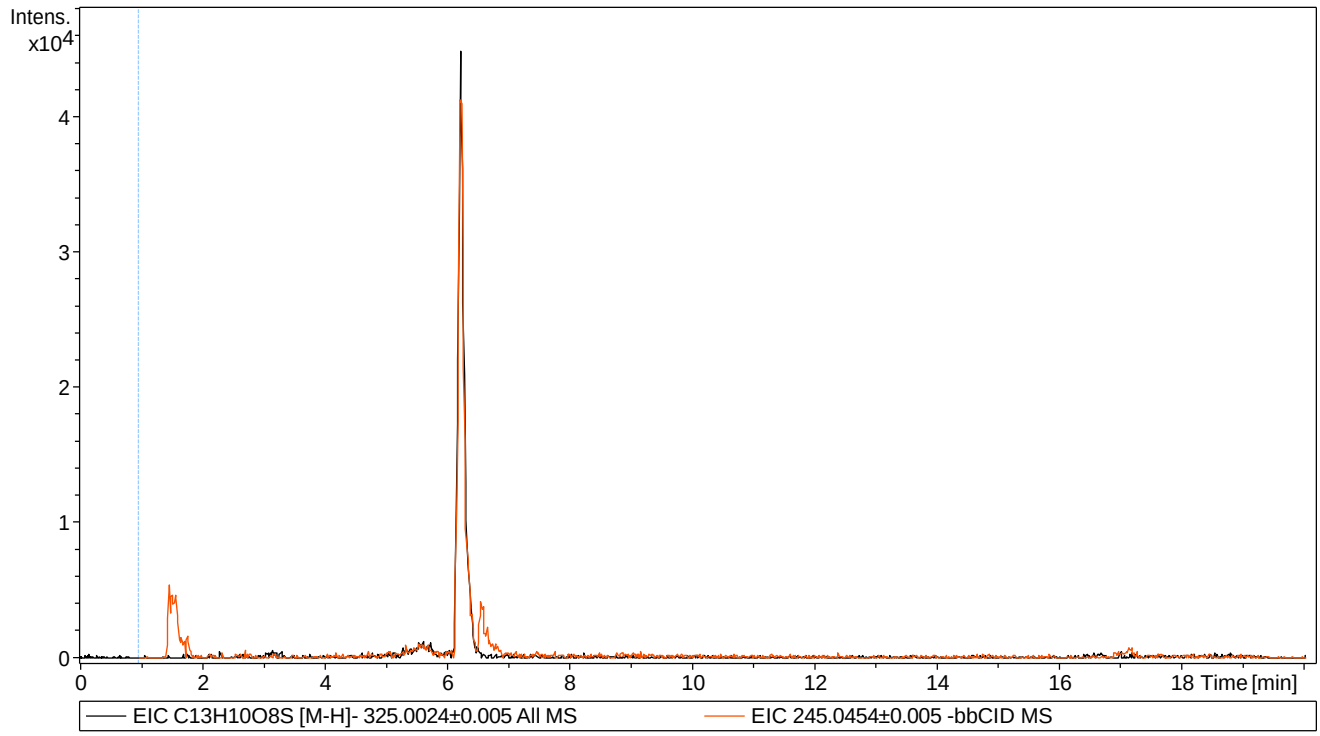
Operator BDAL@DE

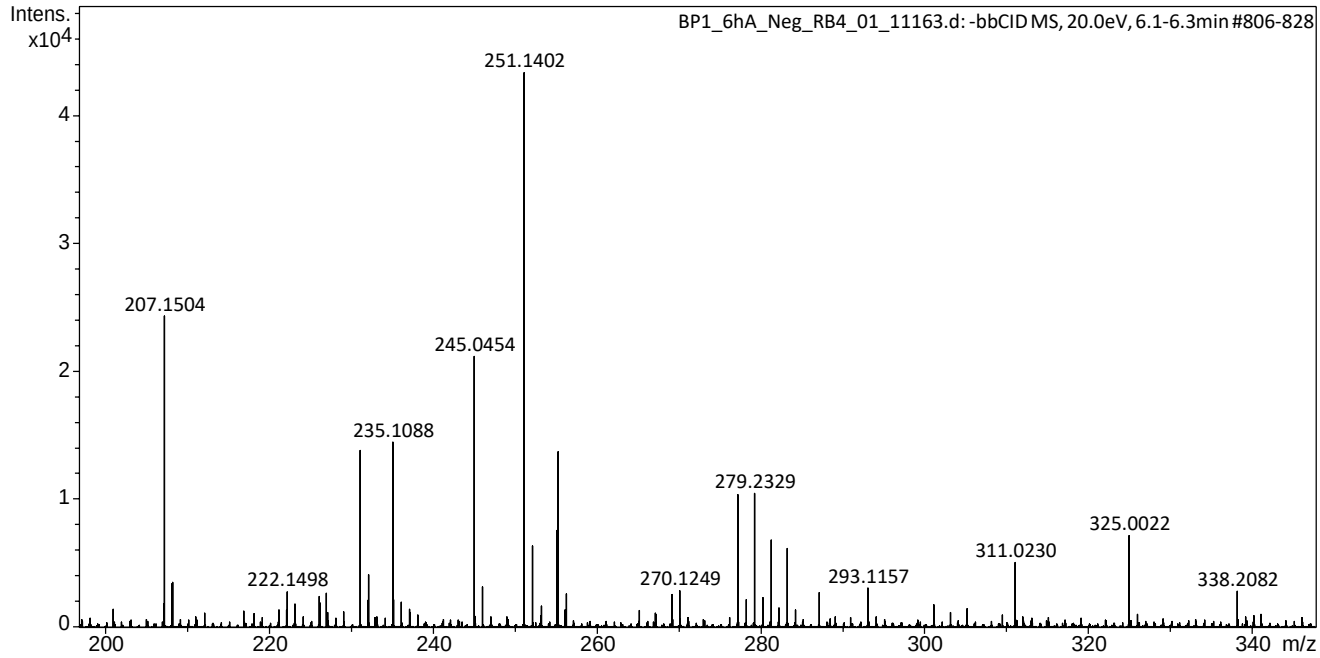
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

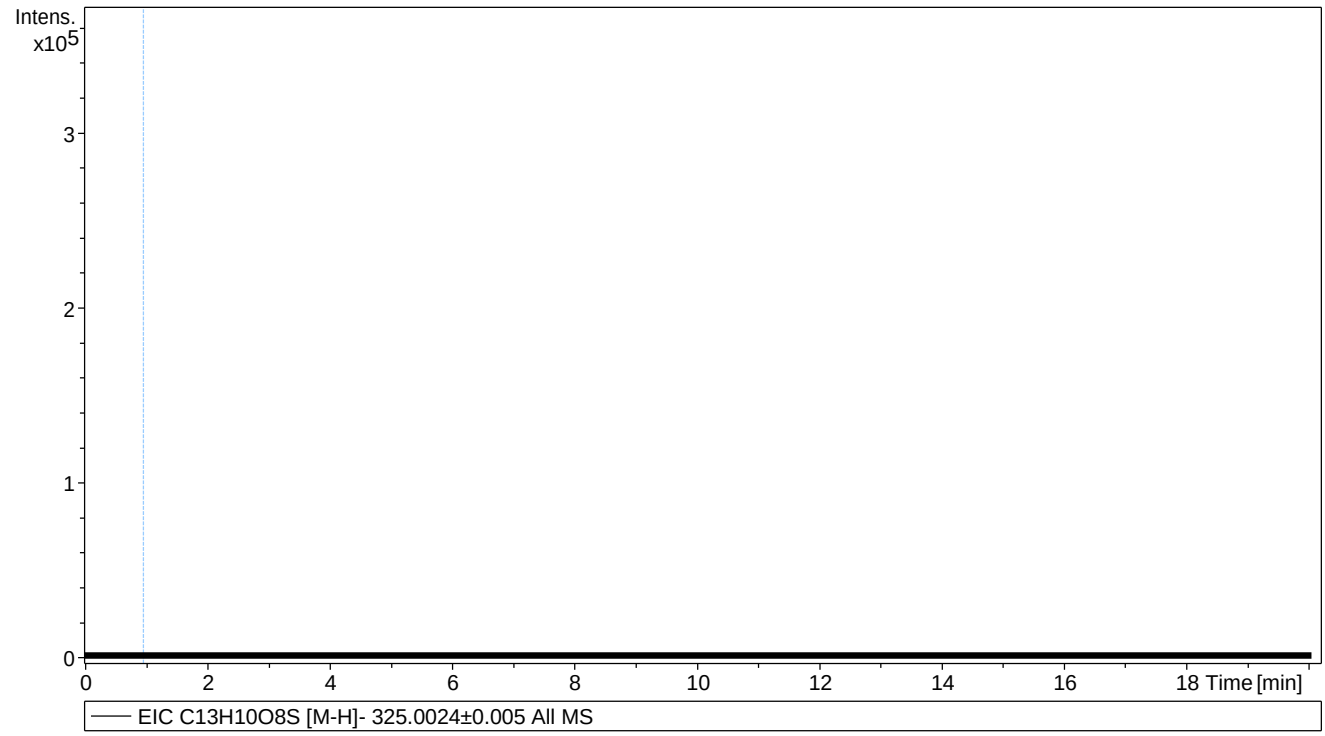
Operator BDAL@DE

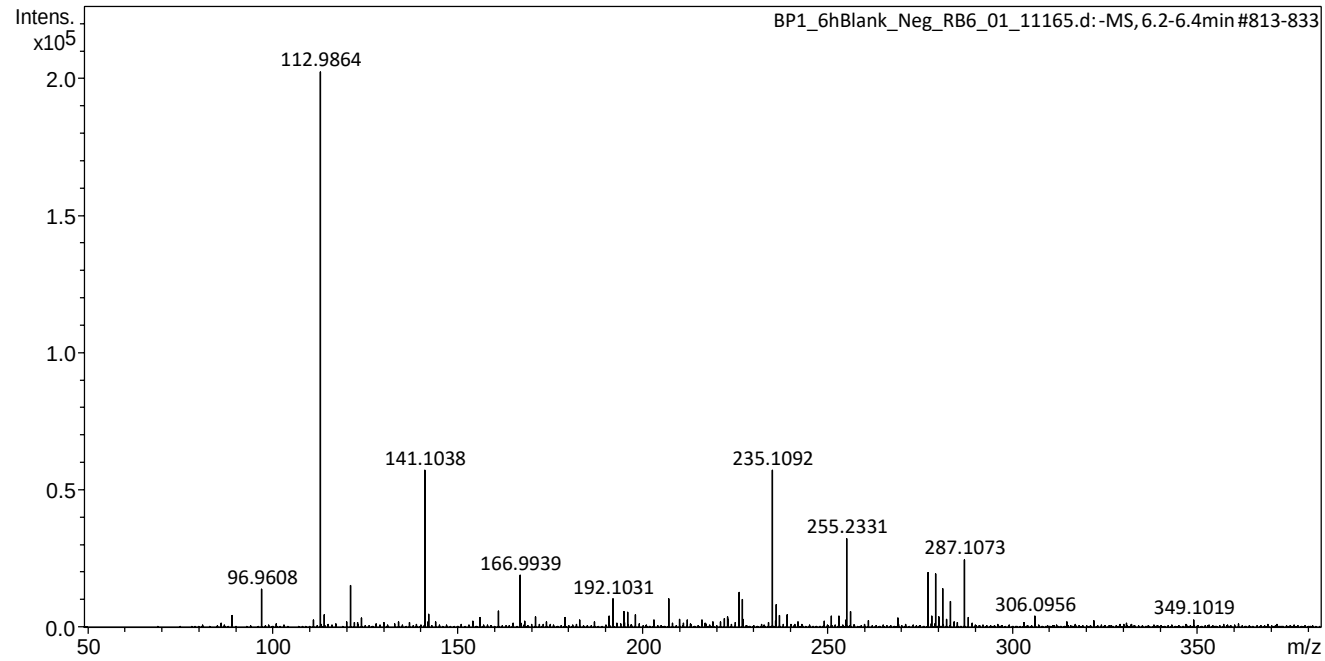
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 23:58:26

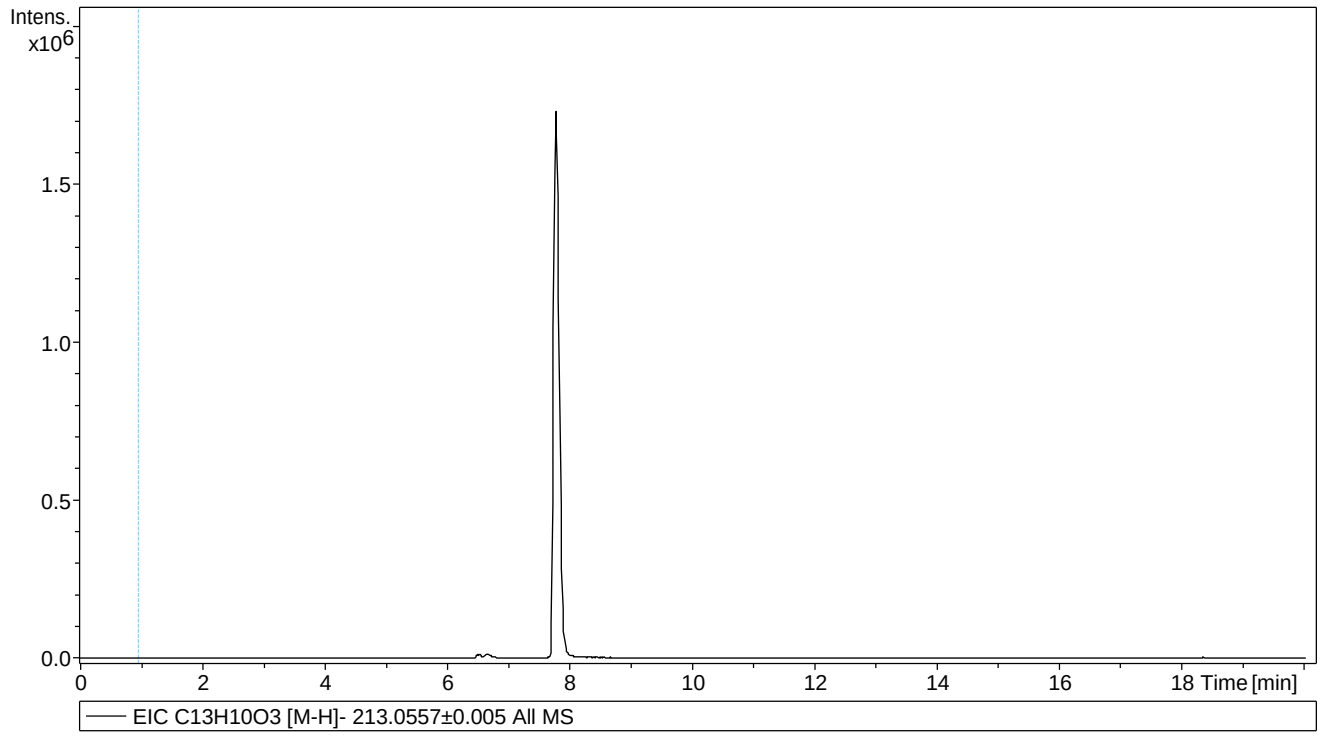
Operator BDAL@DE

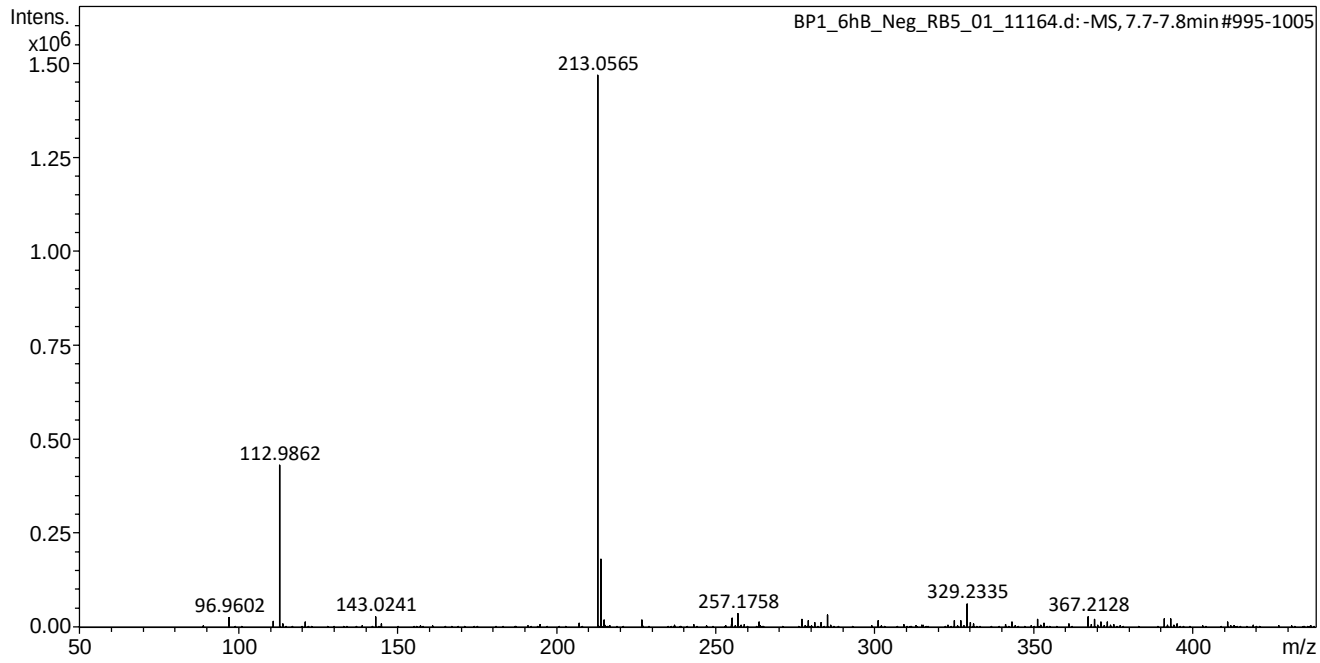
Sample Name BP1_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 23:37:10

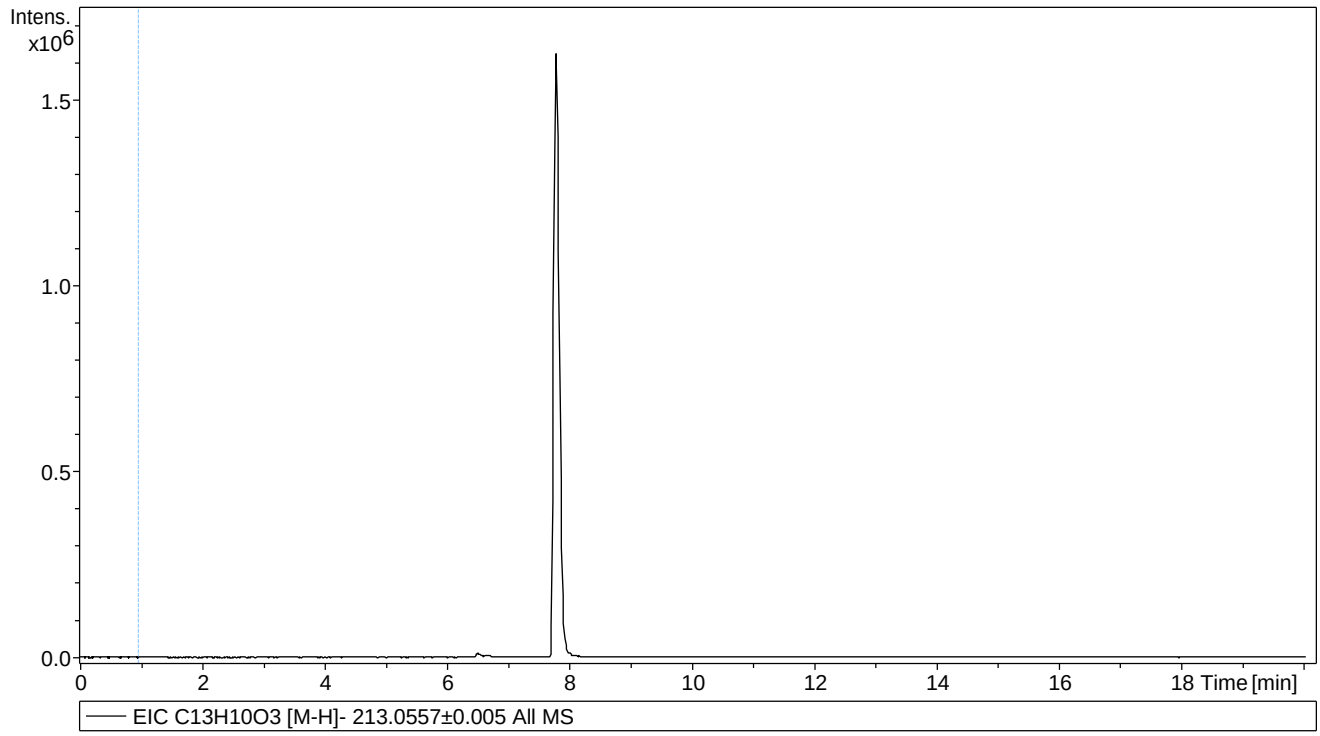
Operator BDAL@DE

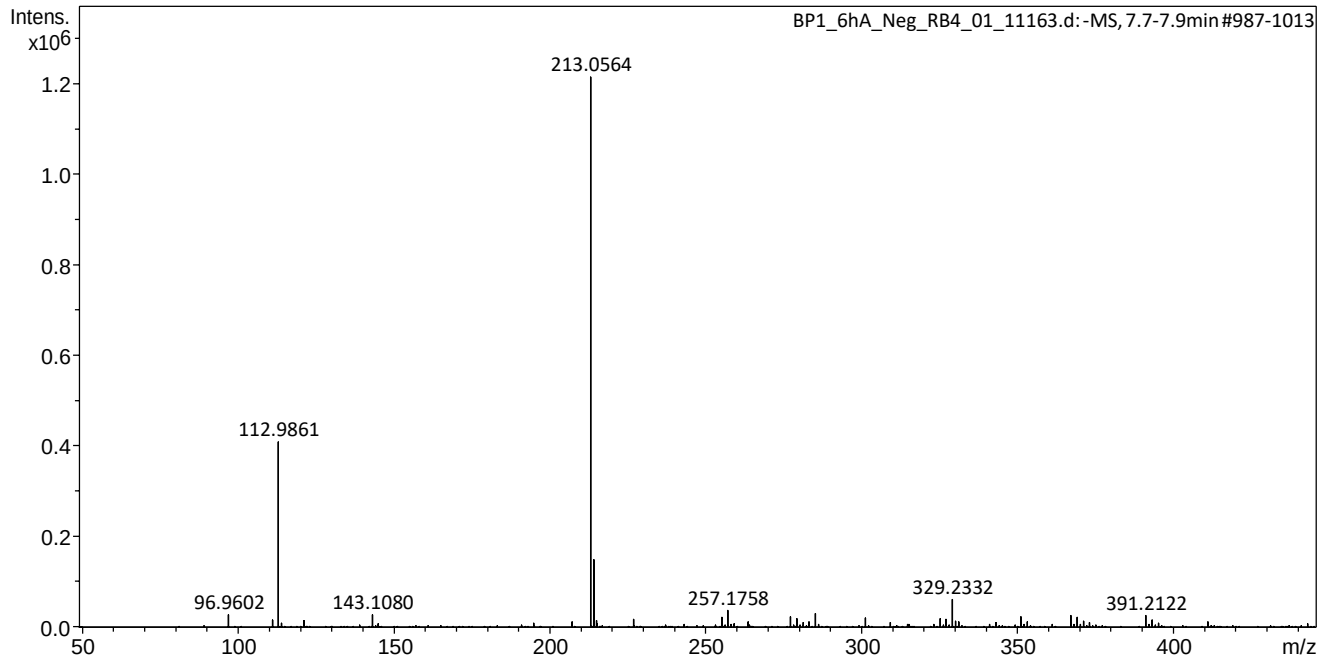
Sample Name BP1_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hA_Neg_RB4_01_11163.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 00:19:42

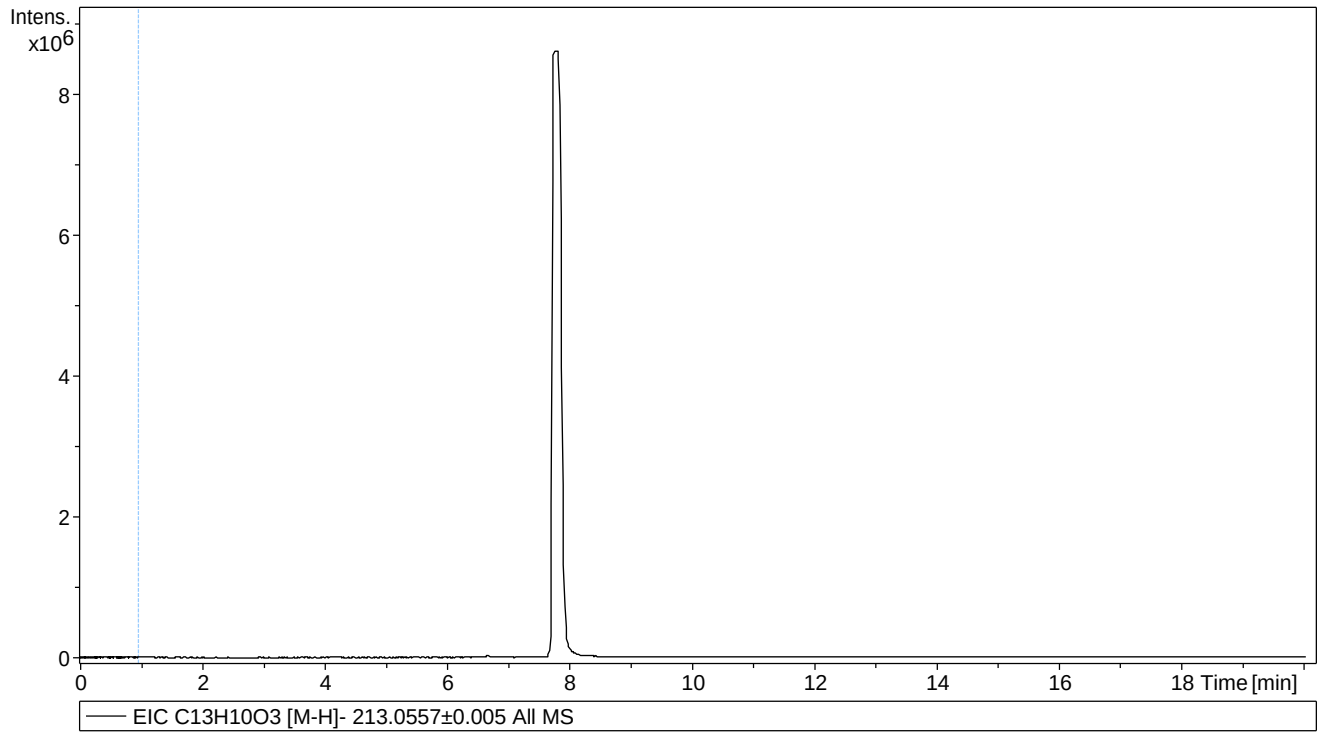
Operator BDAL@DE

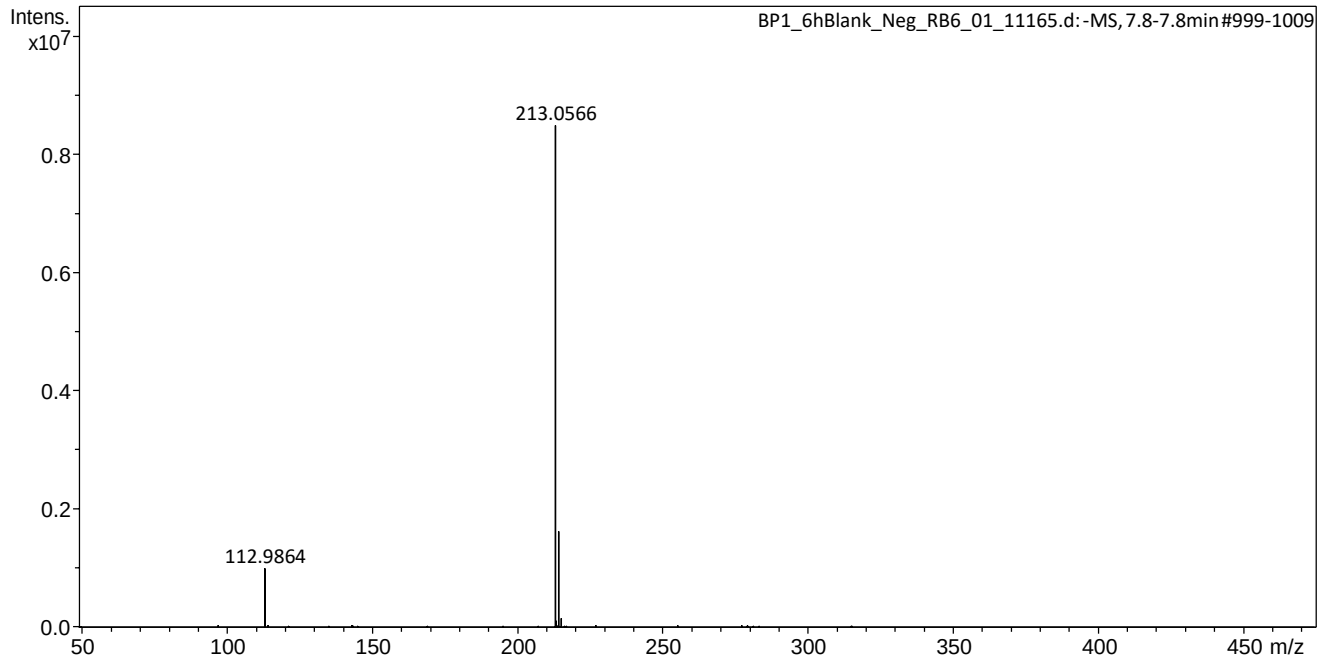
Sample Name BP1_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP1_6hBlank_Neg_RB6_01_11165.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 00:40:59

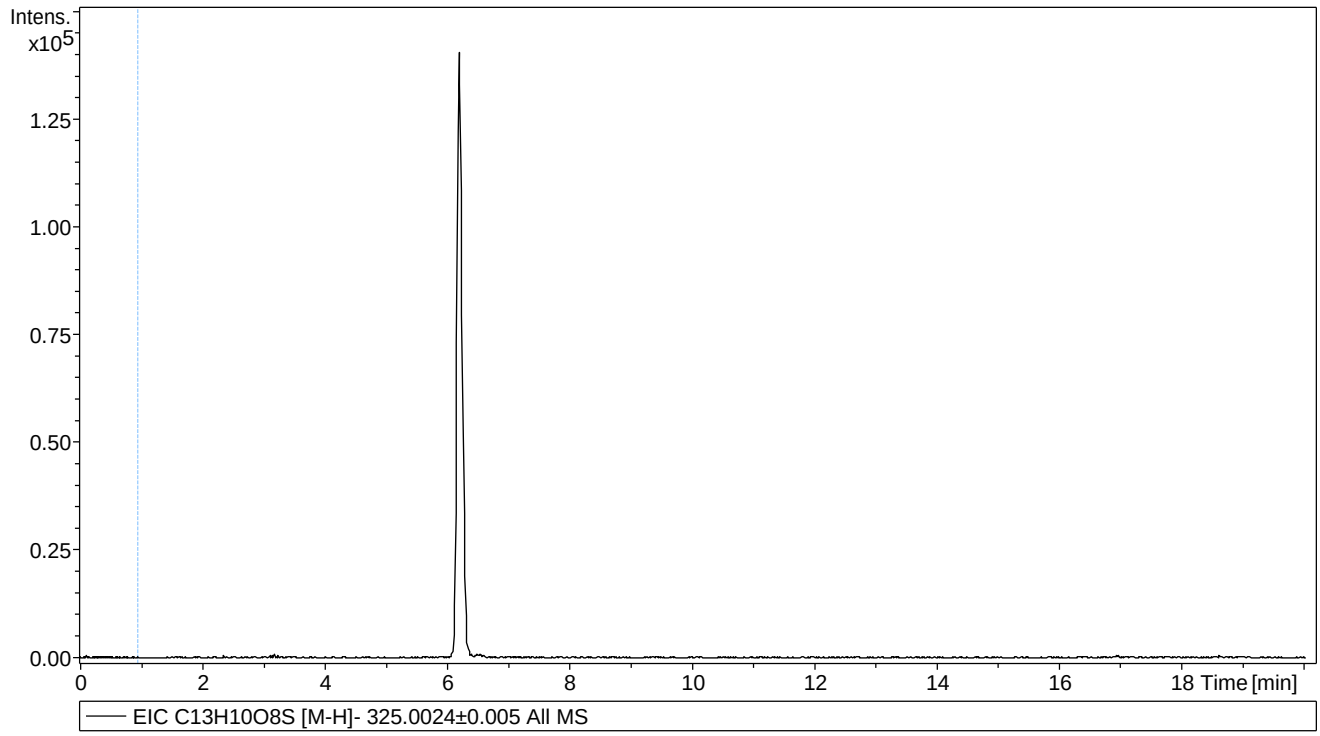
Operator BDAL@DE

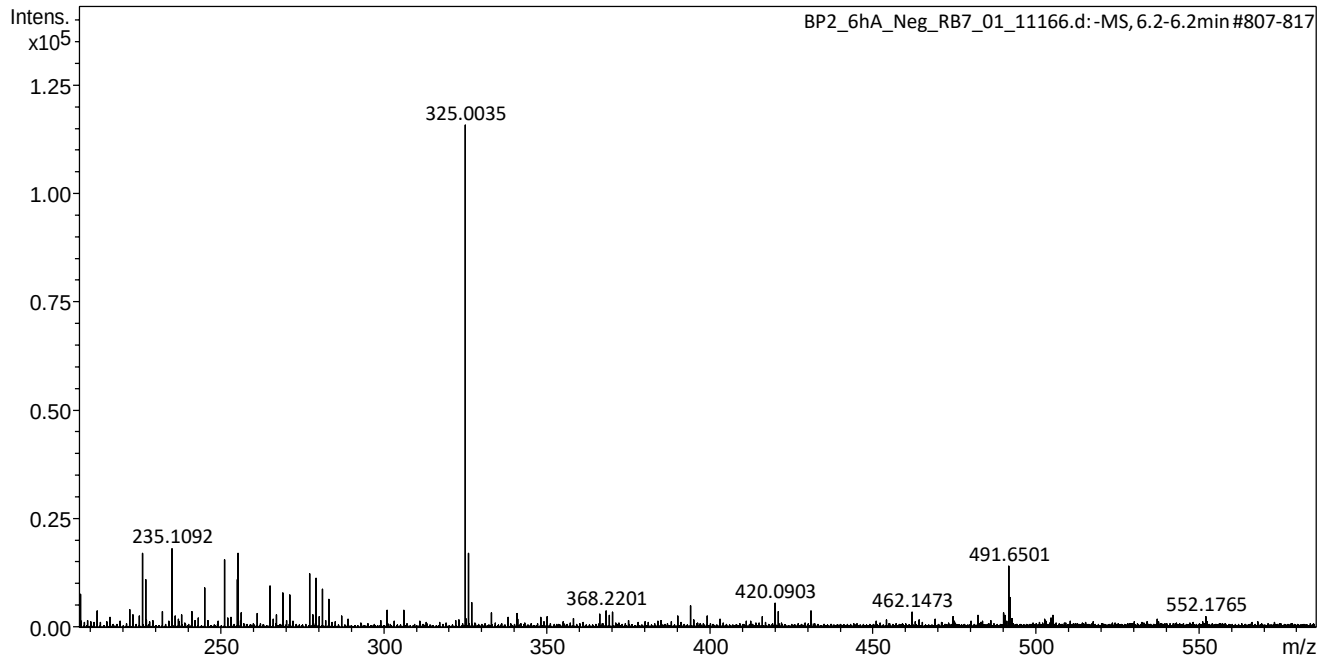
Sample Name BP2_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 00:40:59

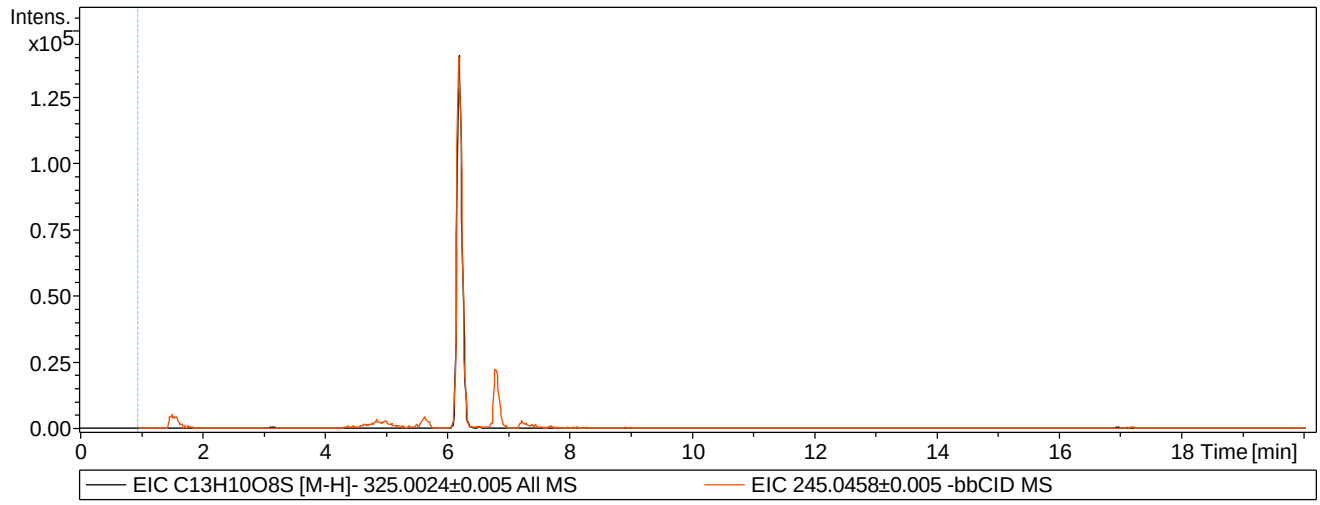
Operator BDAL@DE

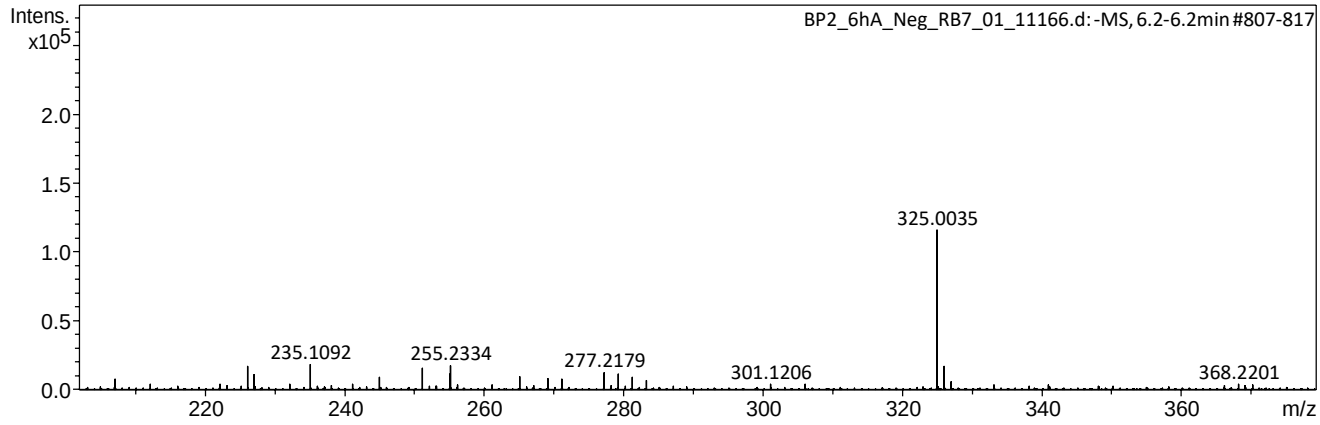
Sample Name BP2_6hA_Neg

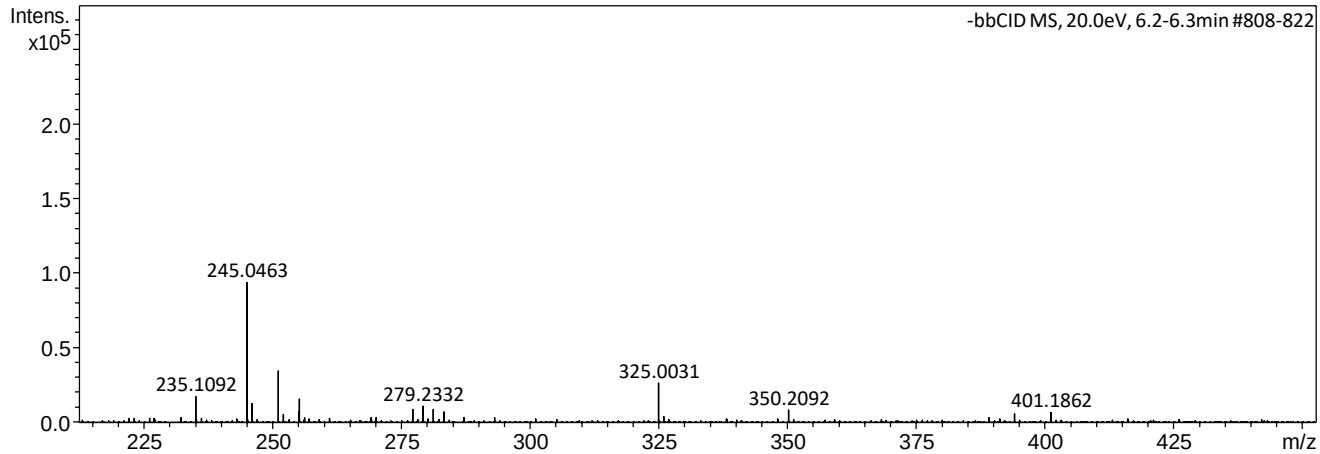
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C







Display Report

Analysis Info

Acquisition Date 15/03/2017 01:02:18

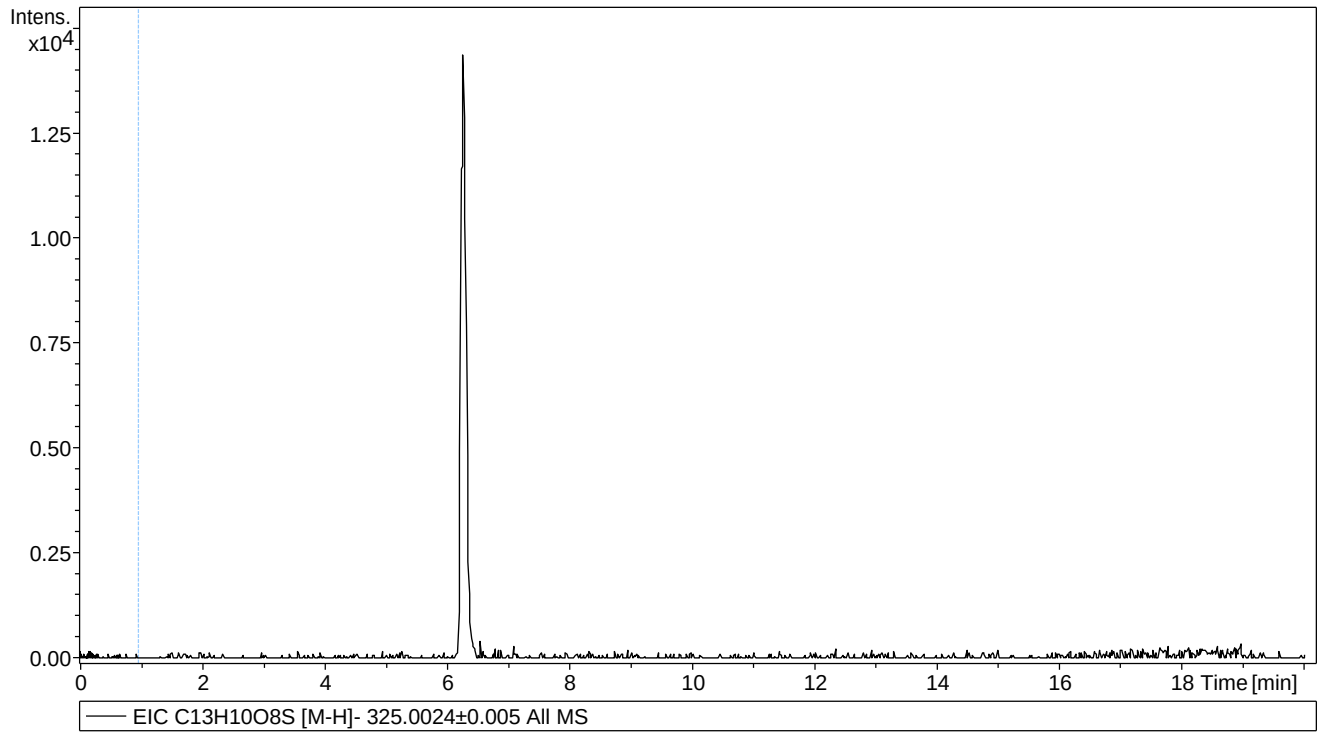
Operator BDAL@DE

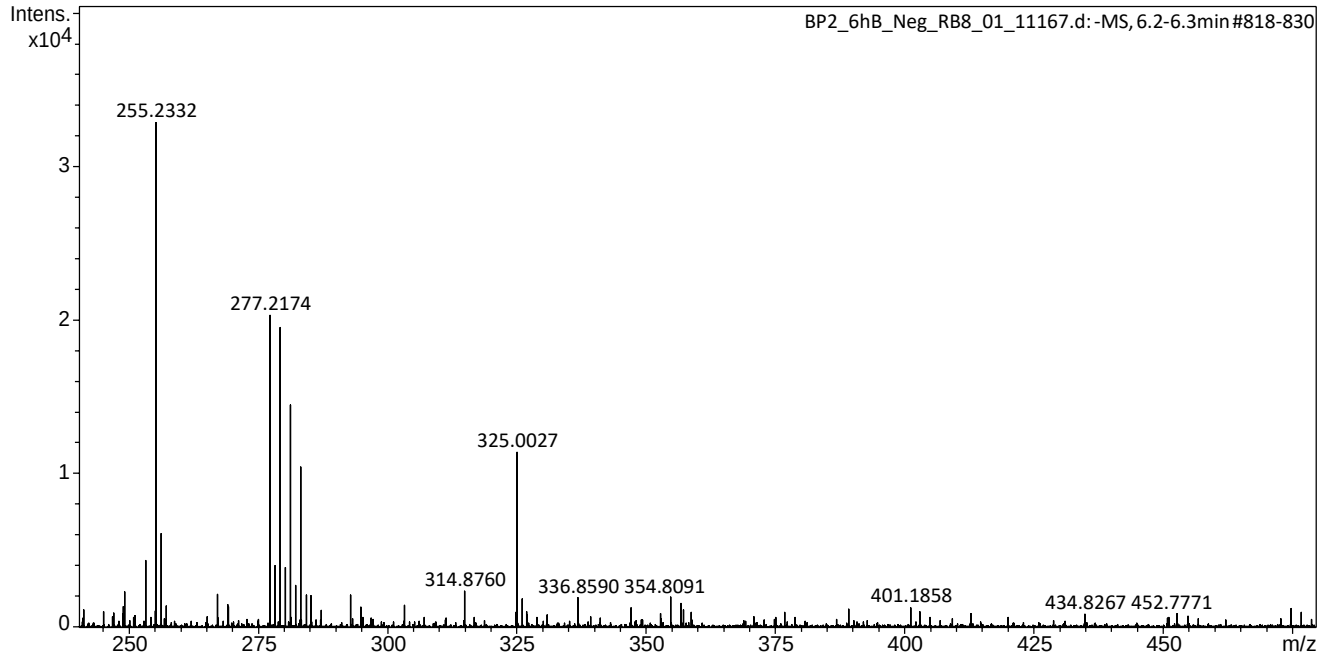
Sample Name BP2_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 15/03/2017 01:02:18

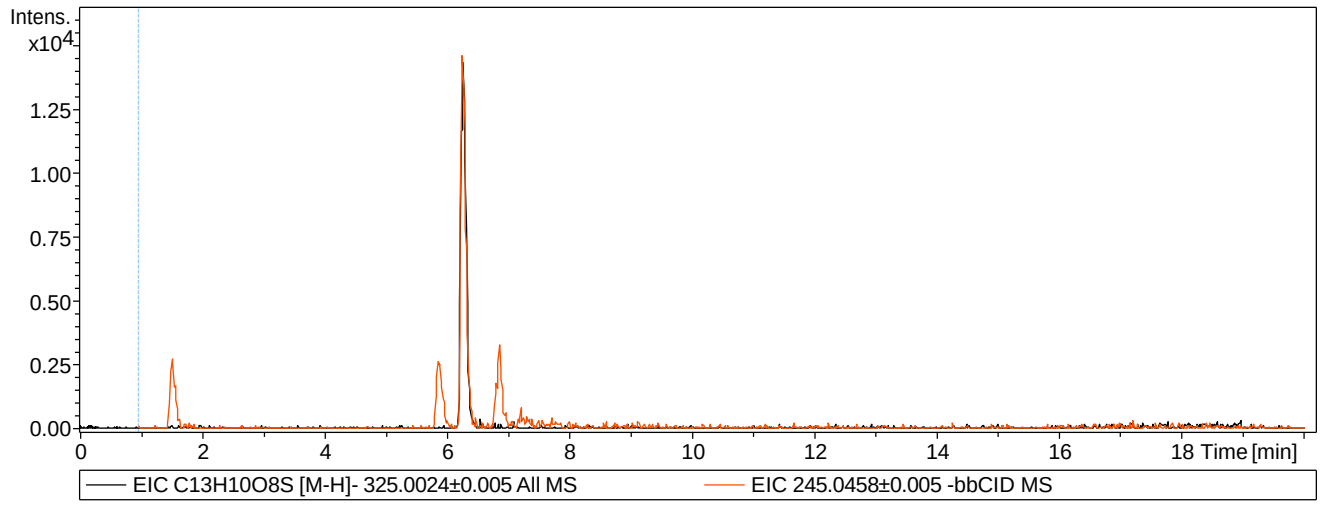
Operator BDAL@DE

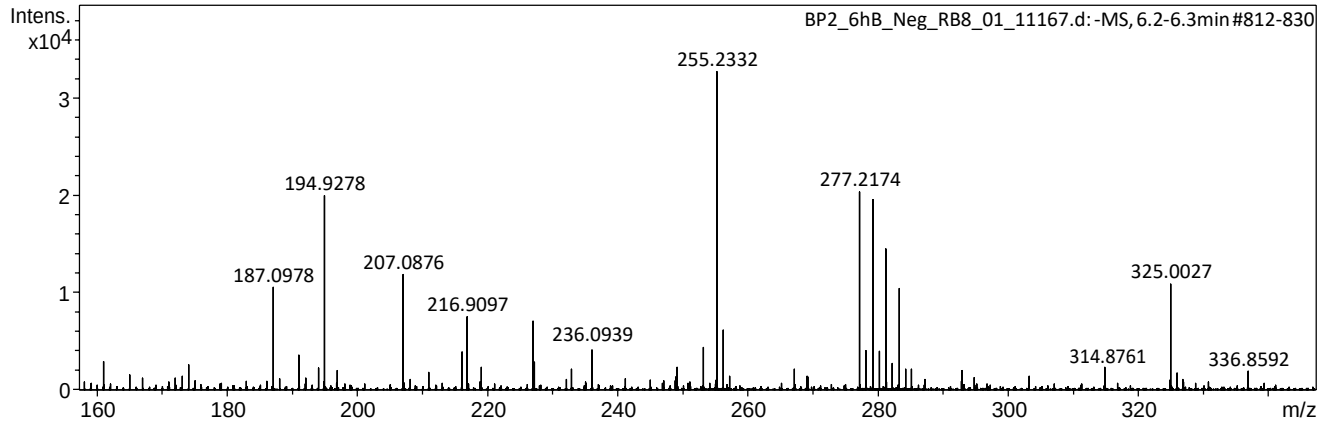
Sample Name BP2_6hB_Neg

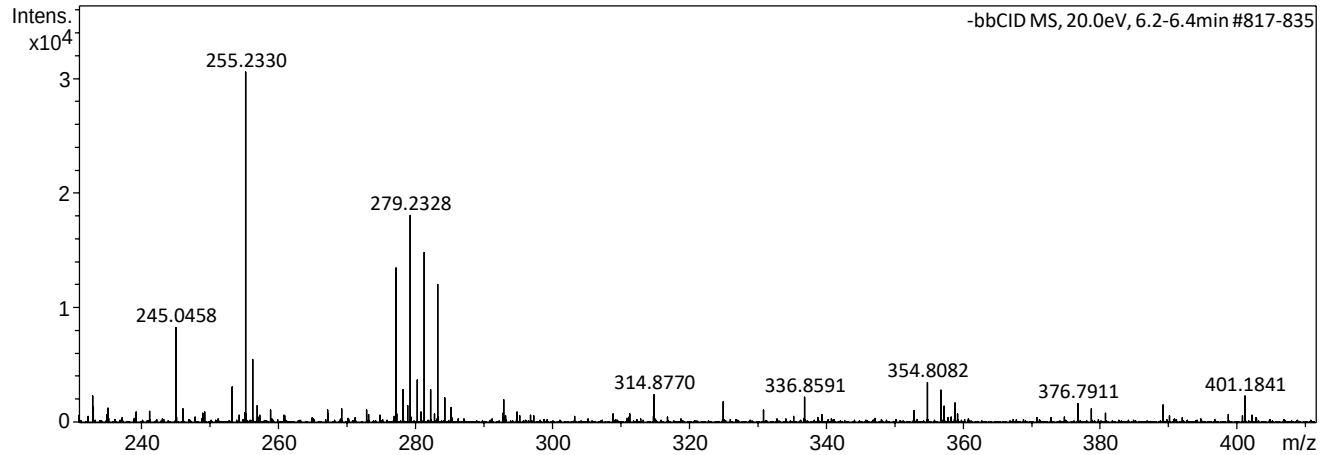
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C







Display Report

Analysis Info

Acquisition Date 15/03/2017 01:23:33

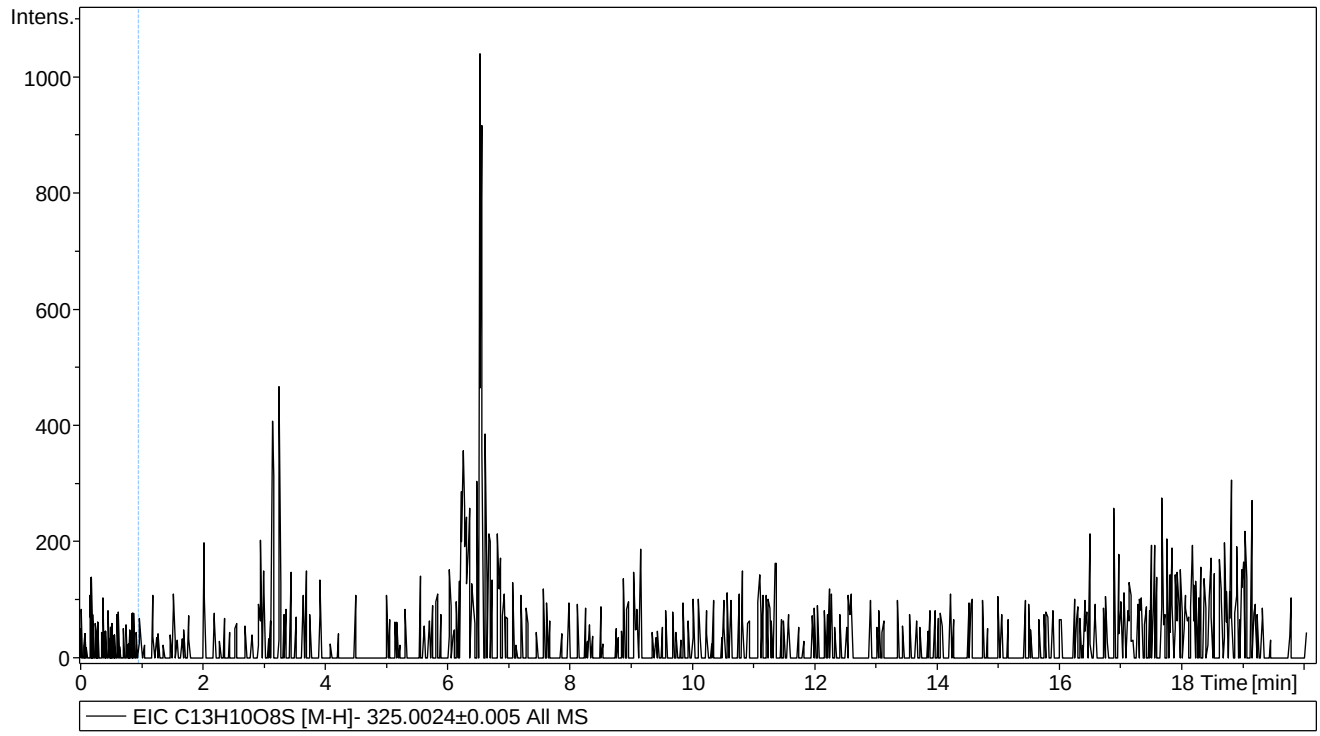
Operator BDAL@DE

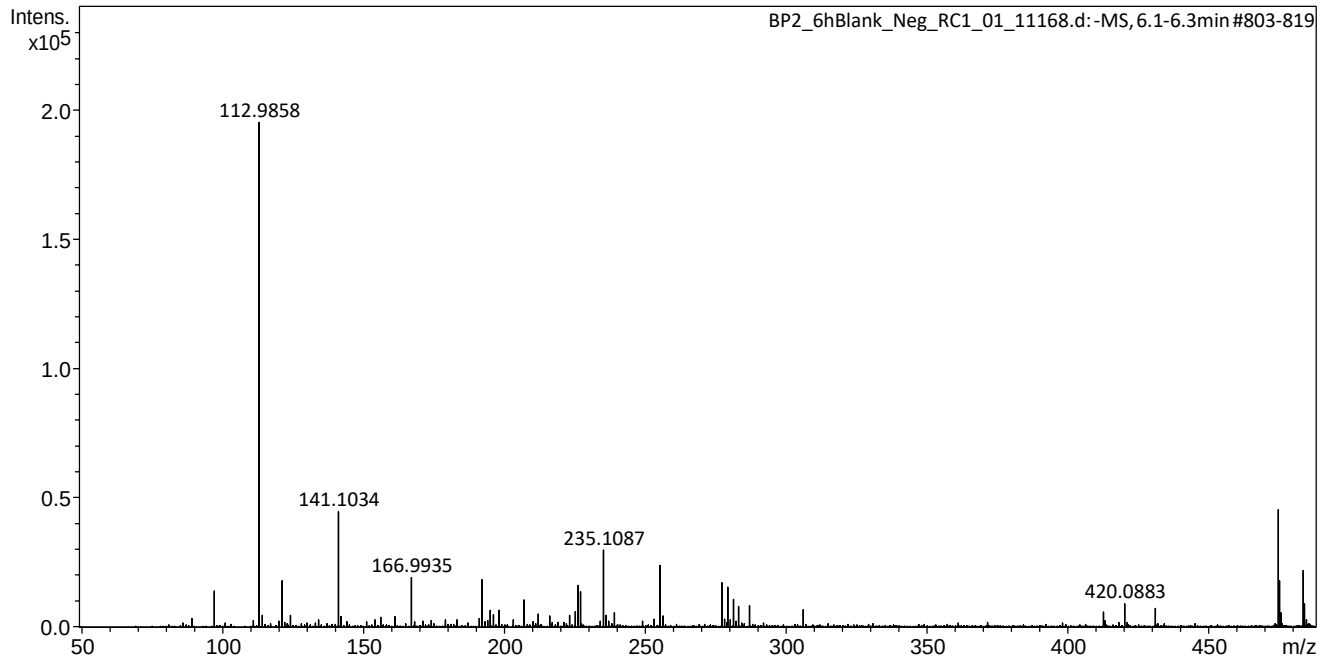
Sample Name BP2_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP2_6hBlank_Neg_RC1_01_11168.d

Display Report

Analysis Info

Acquisition Date 15/03/2017 01:23:33

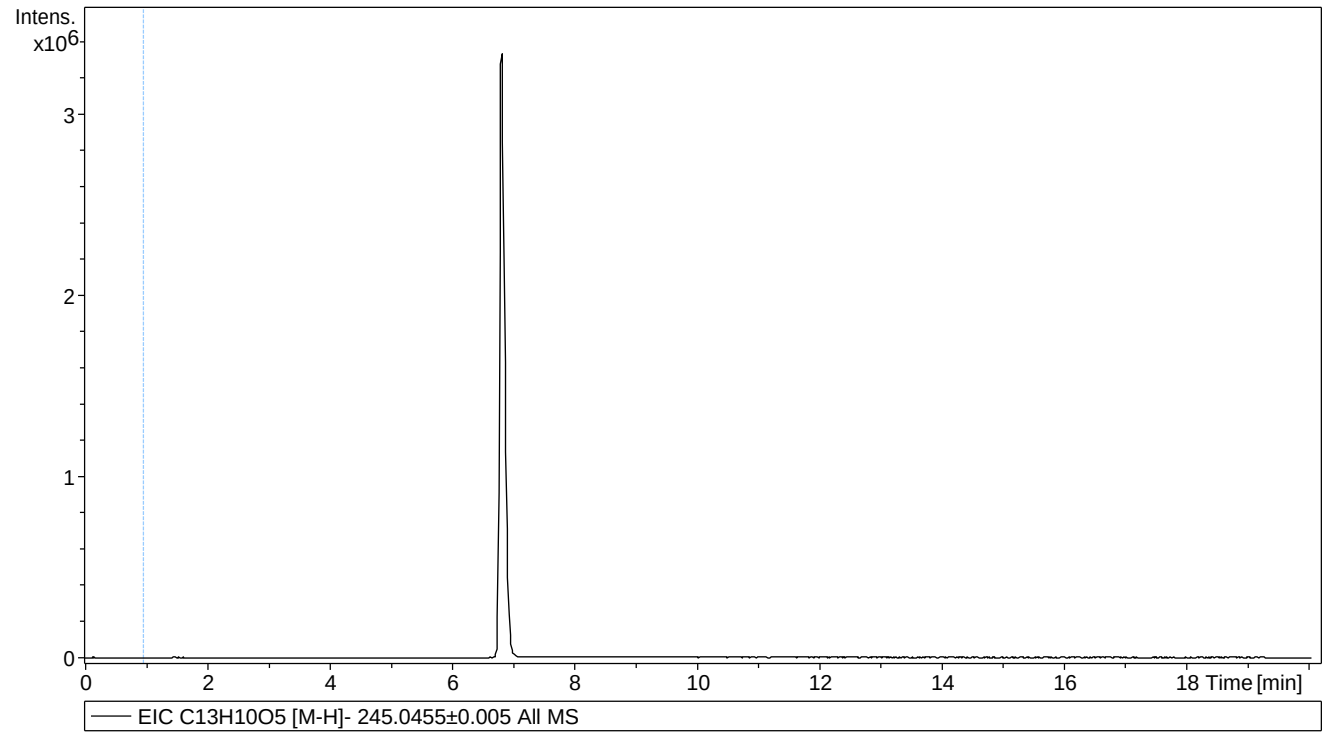
Operator BDAL@DE

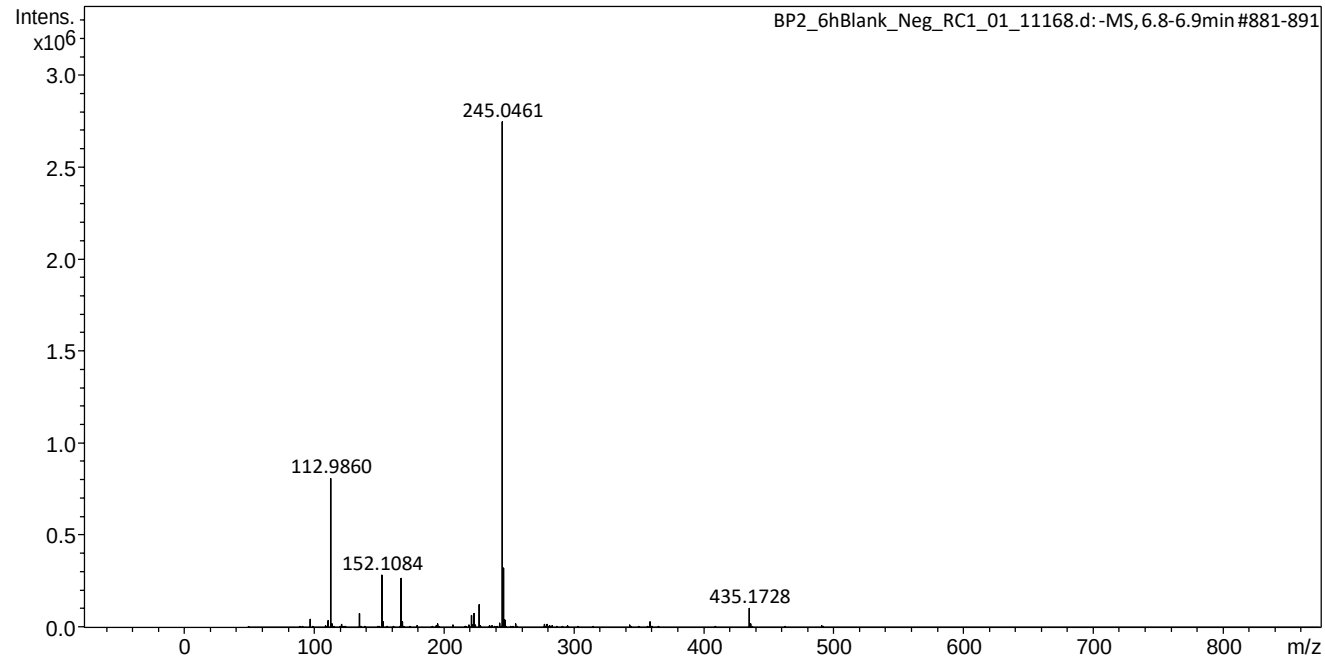
Sample Name BP2_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





BP2_6hBlank_Neg_RC1_01_11168.d

Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

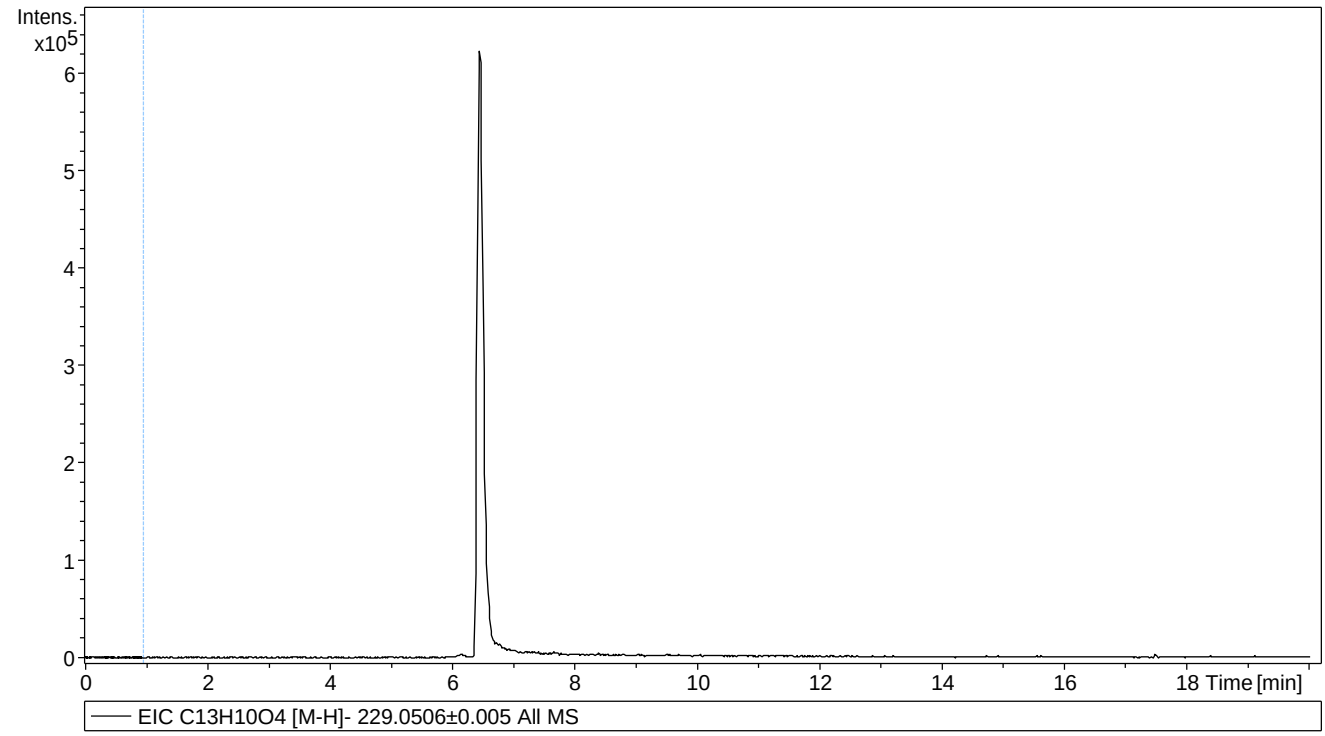
Operator BDAL@DE

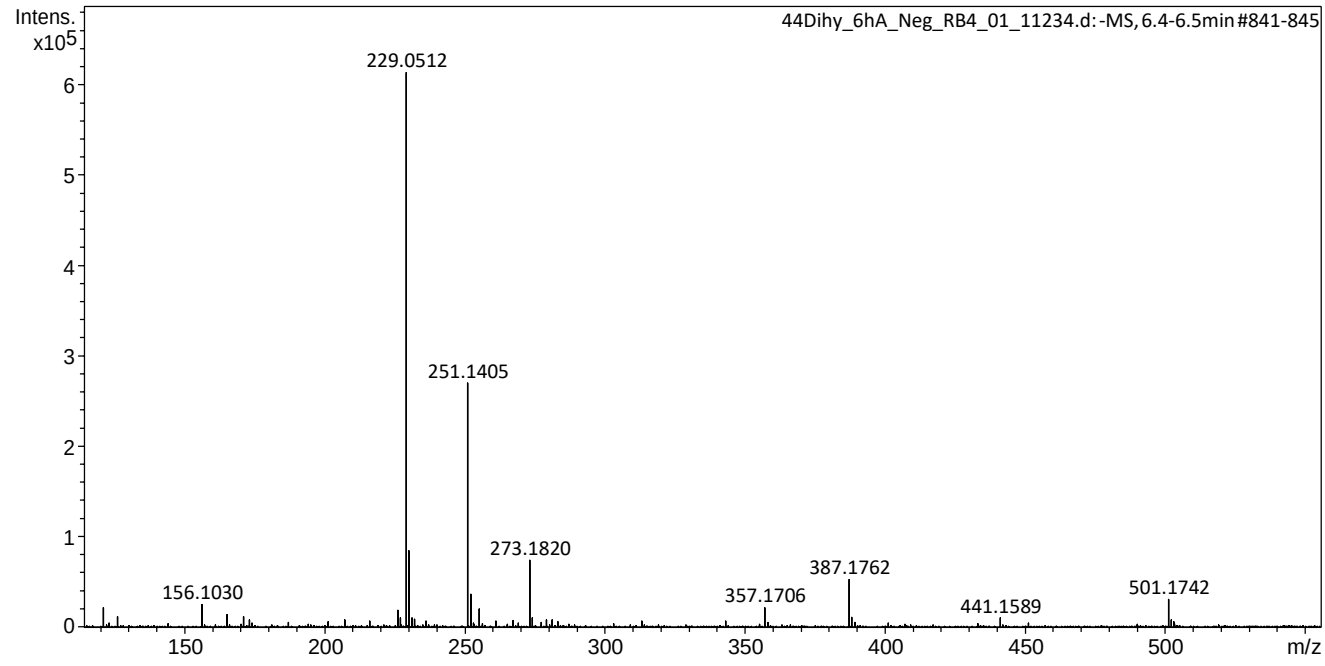
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

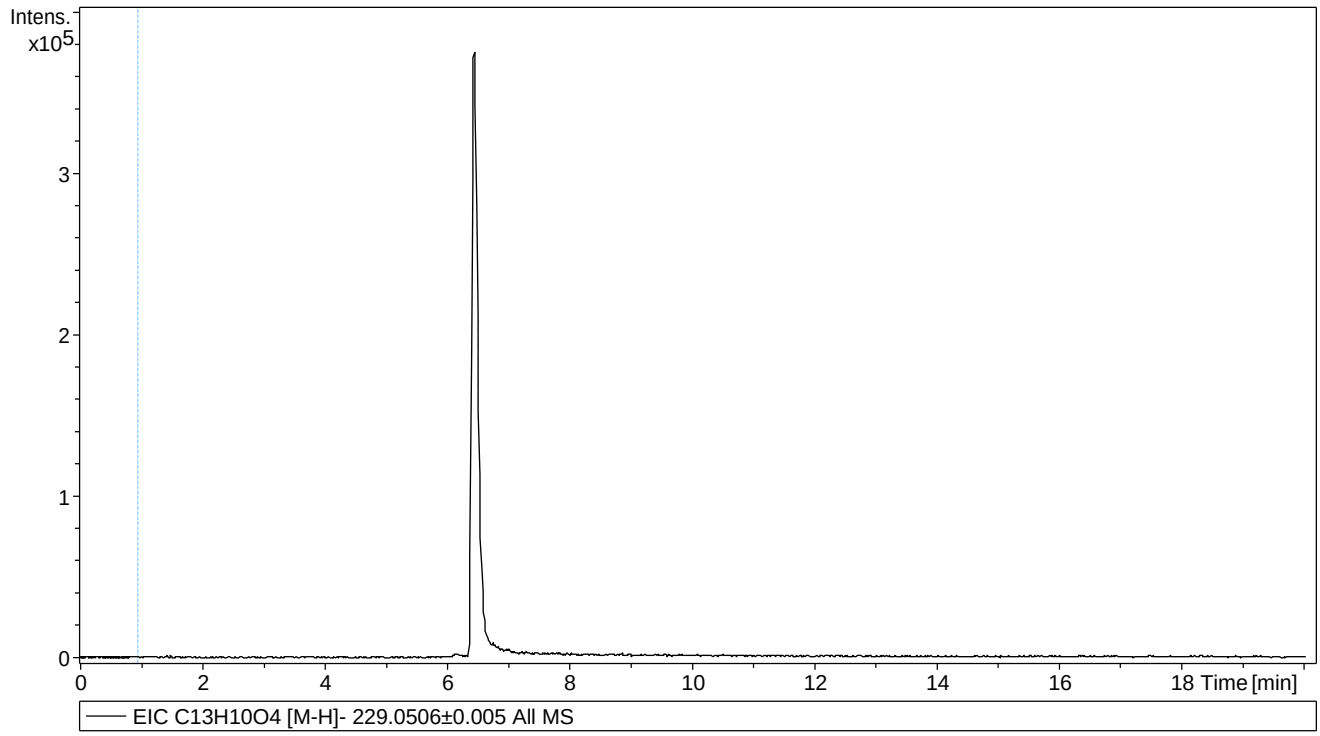
Operator BDAL@DE

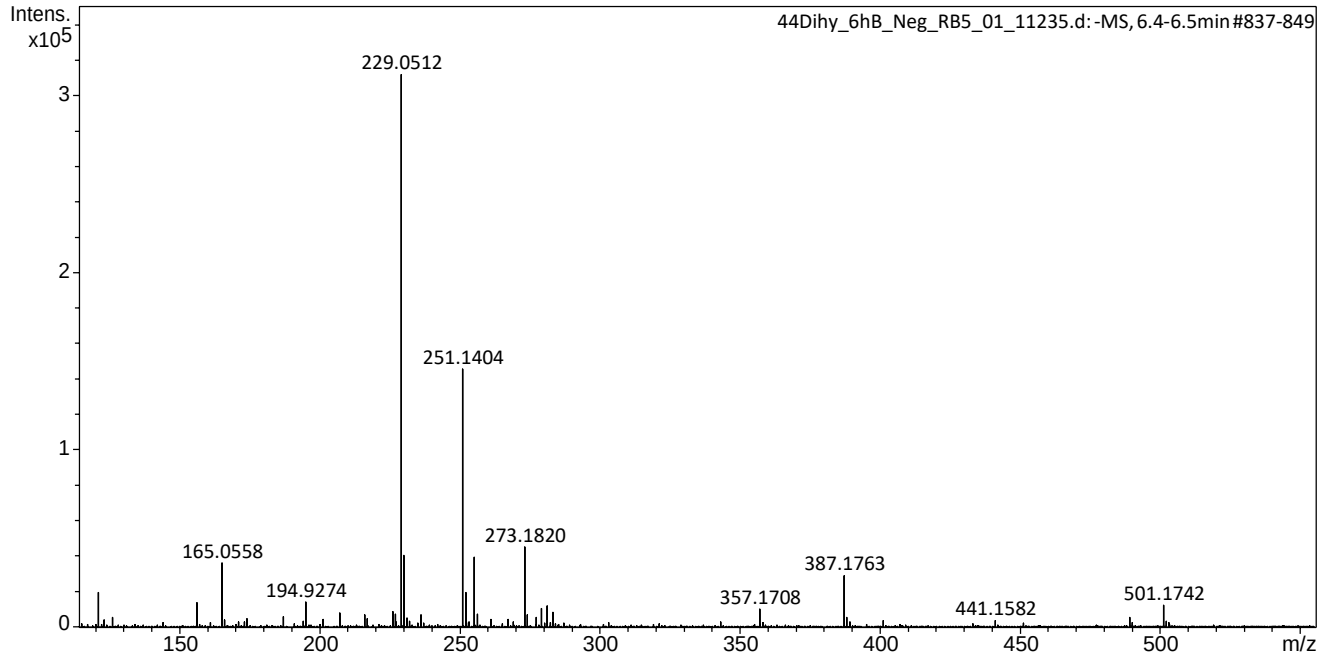
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 07/08/2017 14:55:33

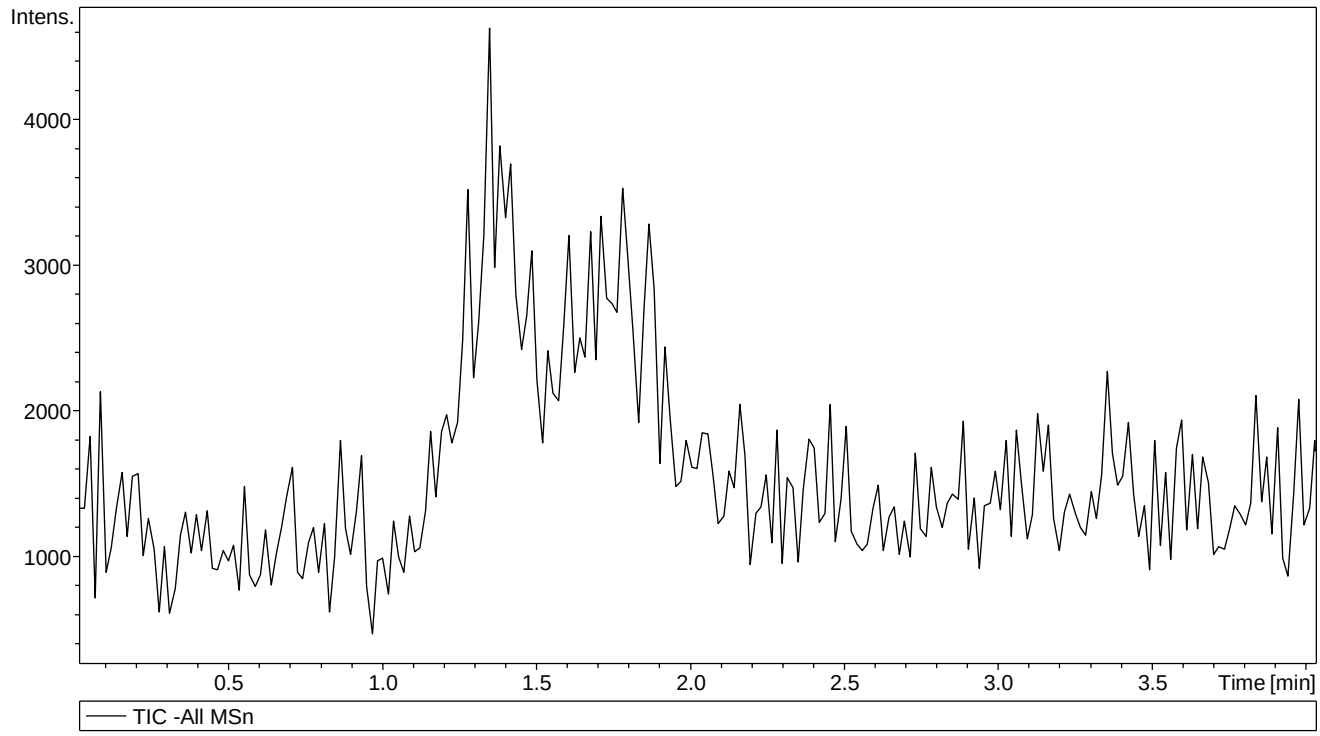
Operator BDAL@DE

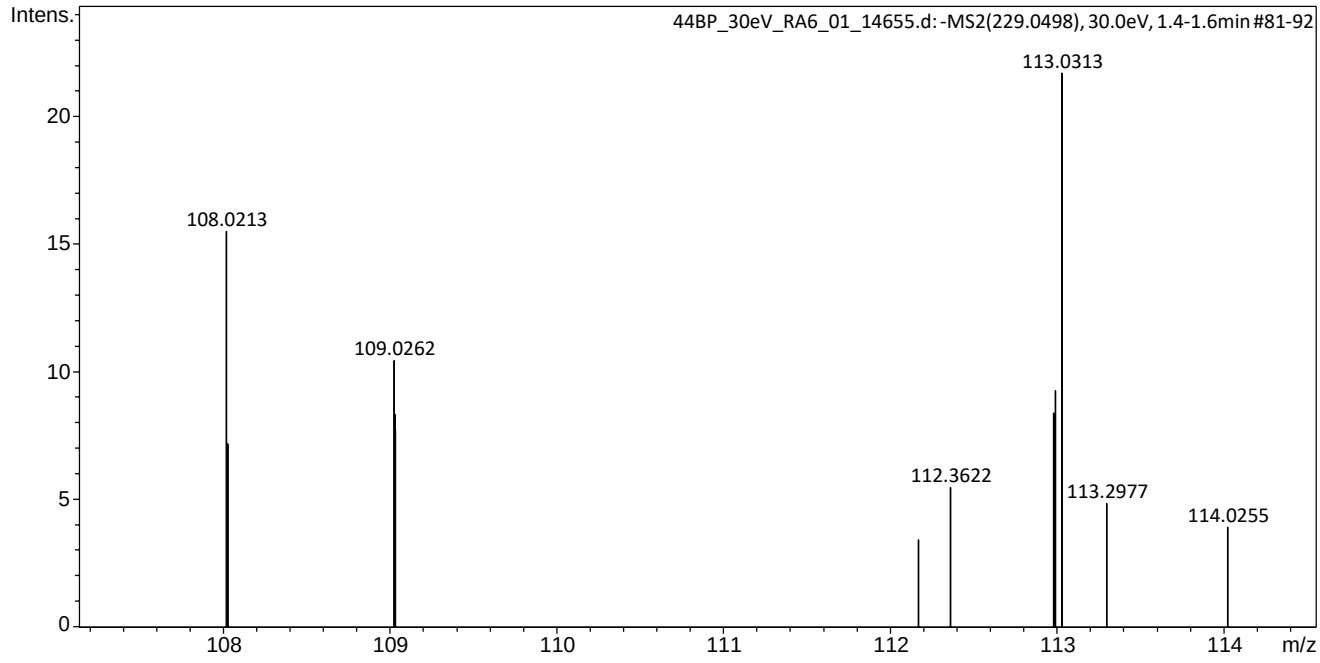
Sample Name 44BP_30eV

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	75 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:40:08

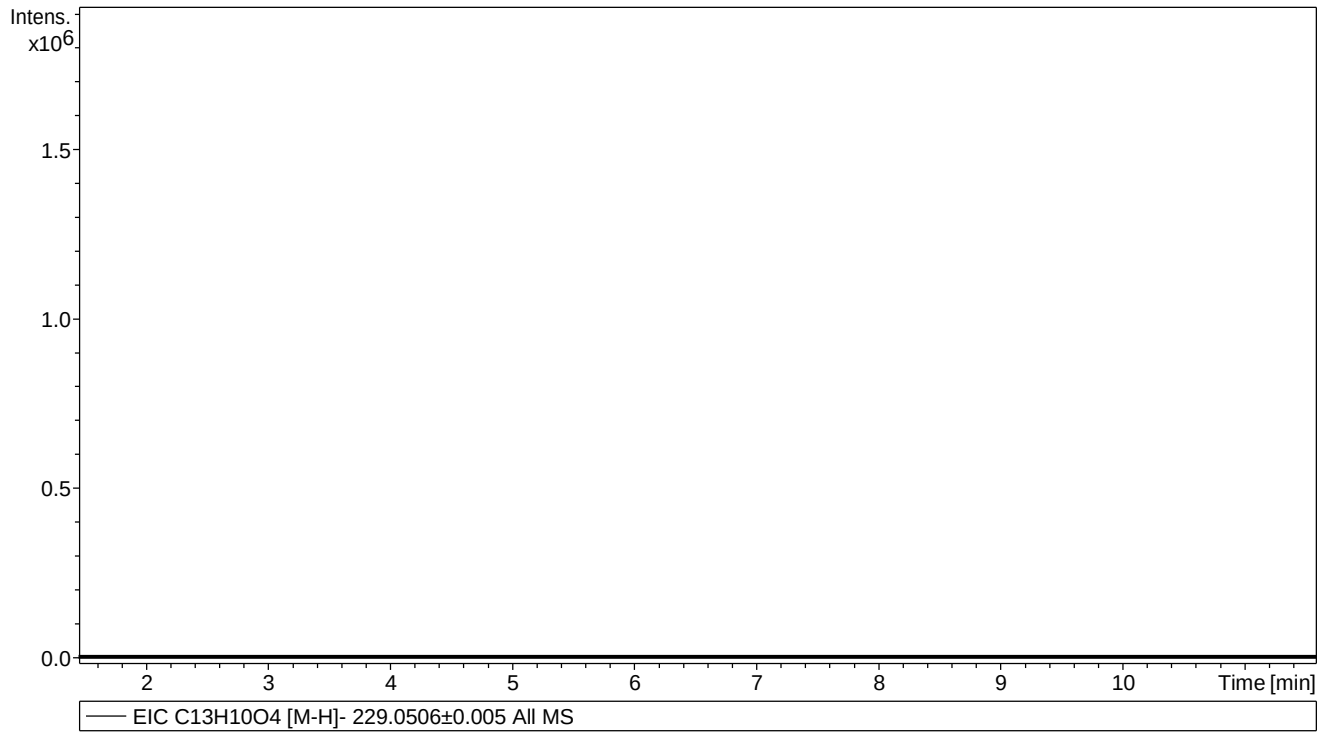
Operator BDAL@DE

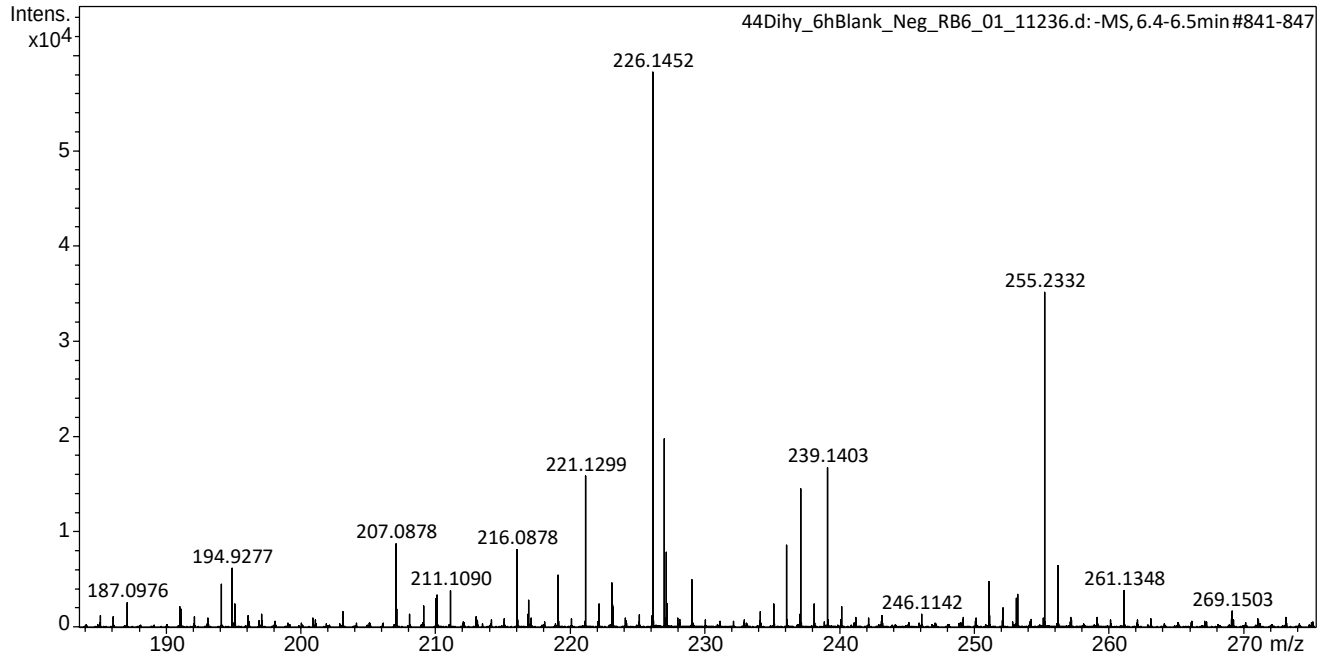
Sample Name 44Dihy_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

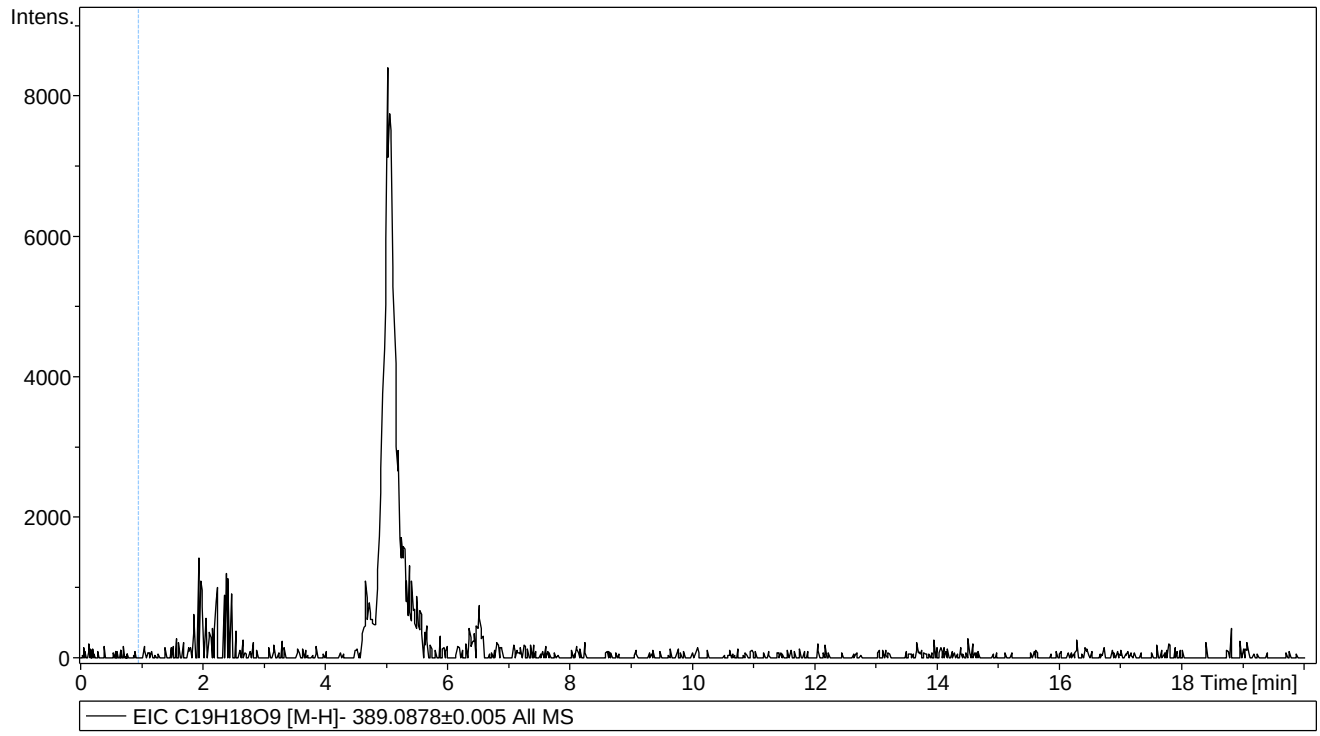
Operator BDAL@DE

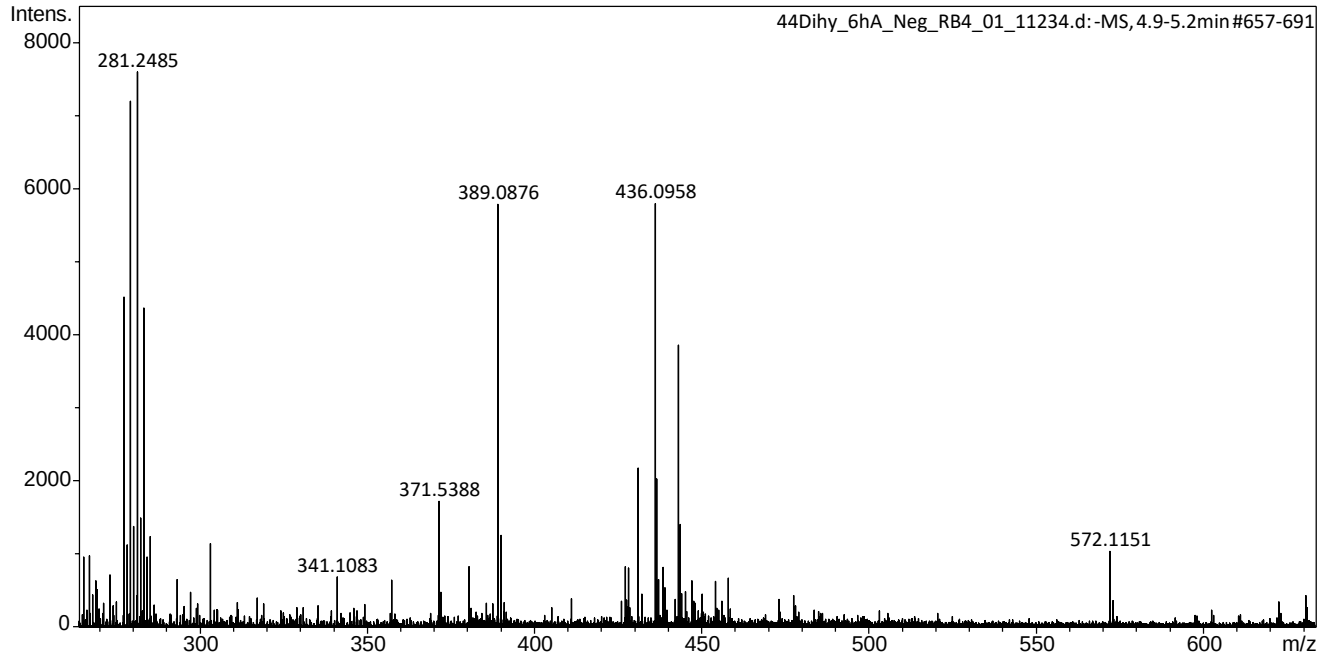
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

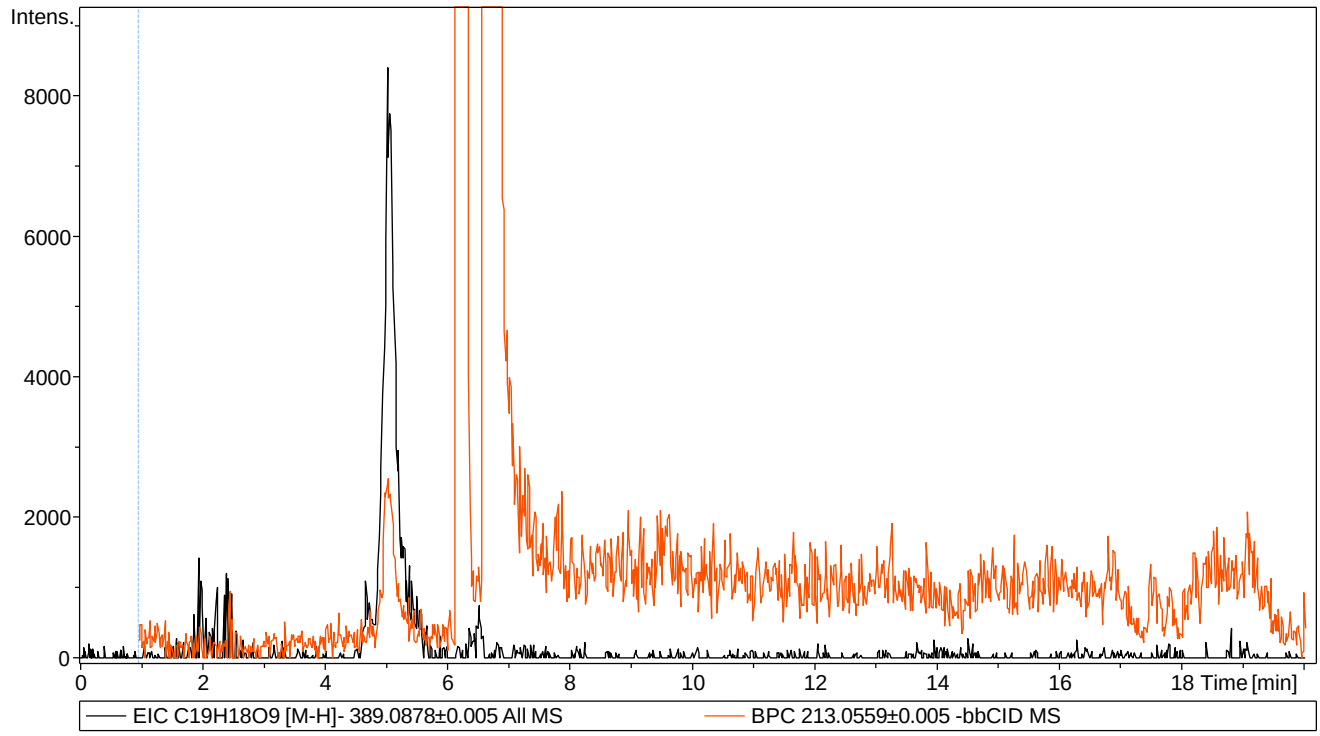
Operator BDAL@DE

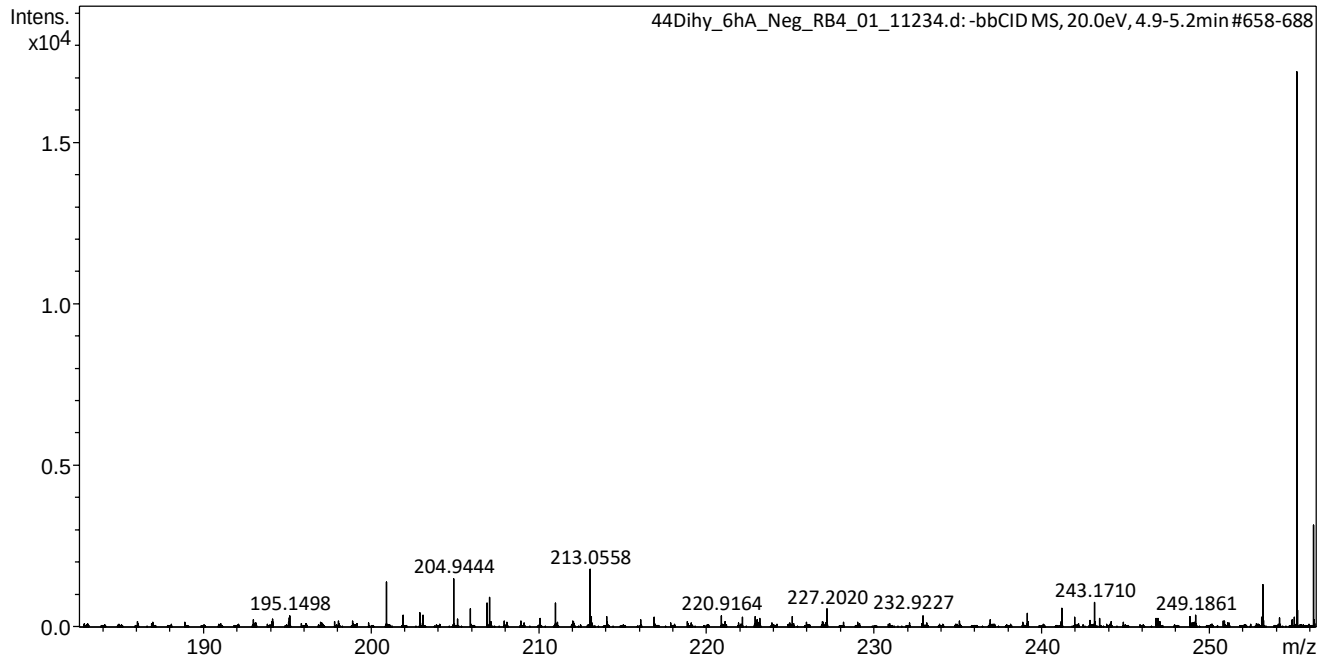
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

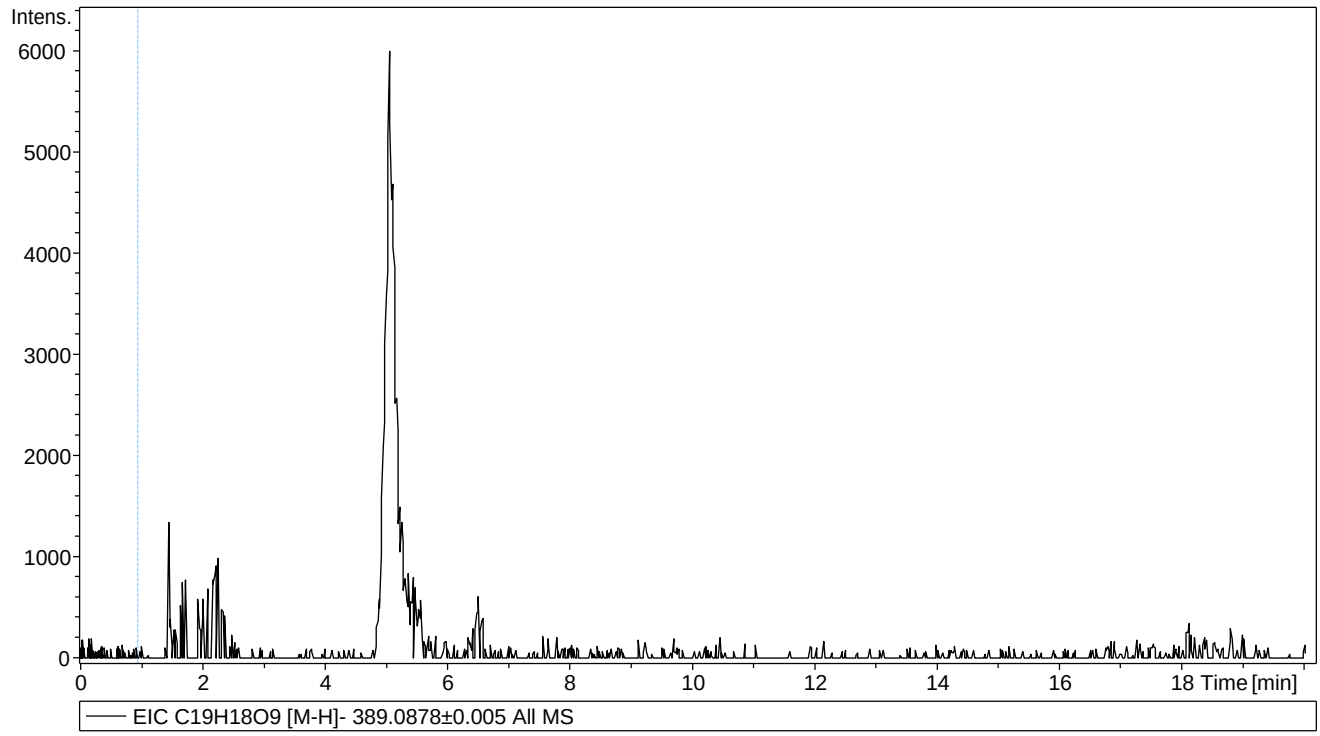
Operator BDAL@DE

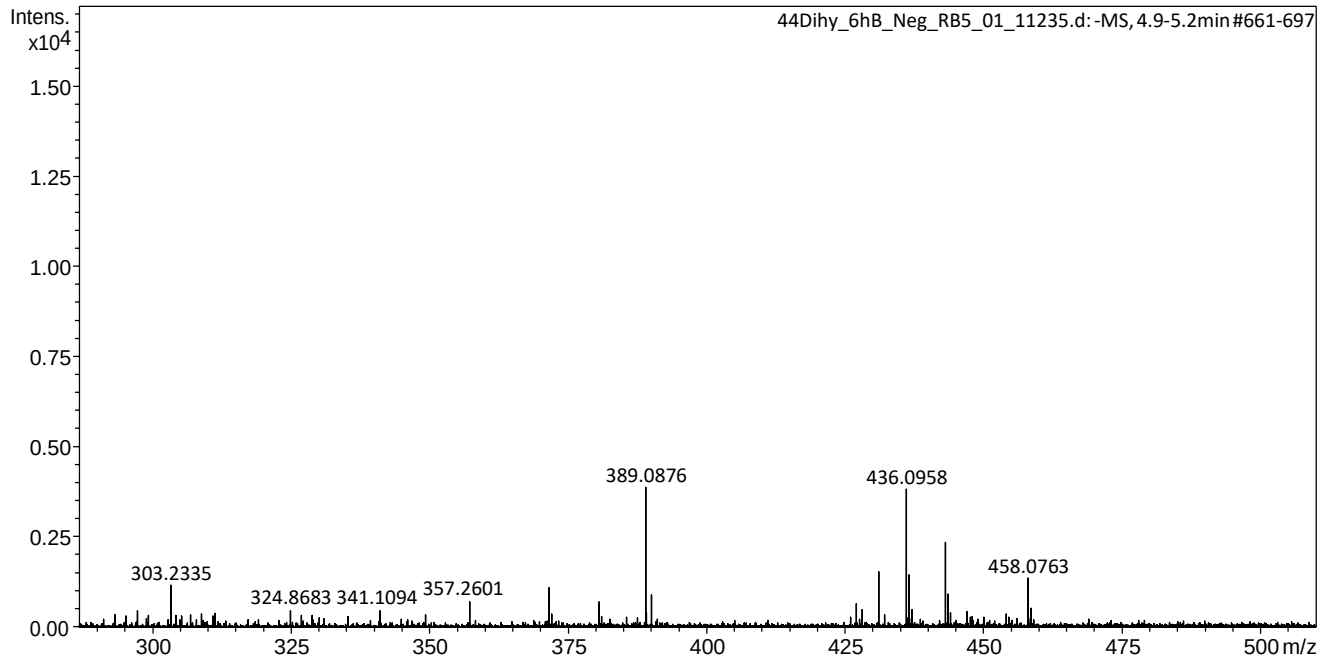
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

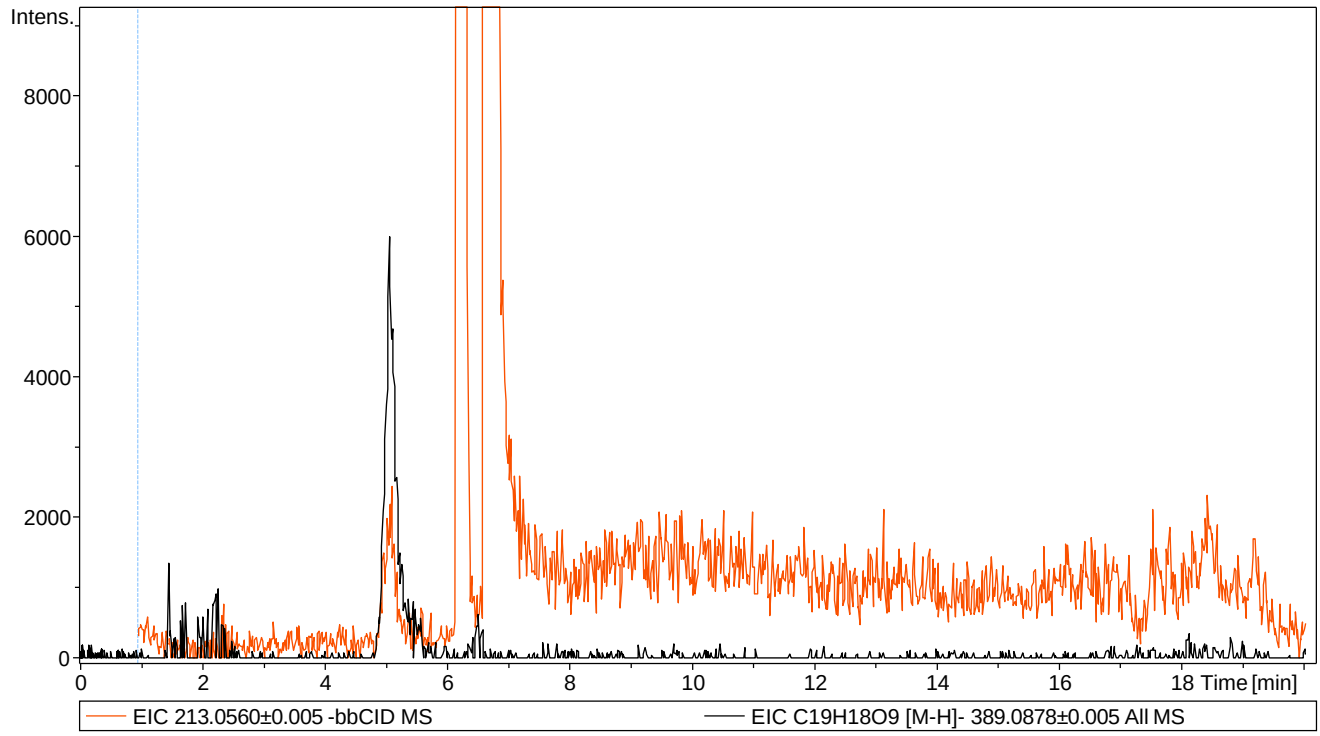
Operator BDAL@DE

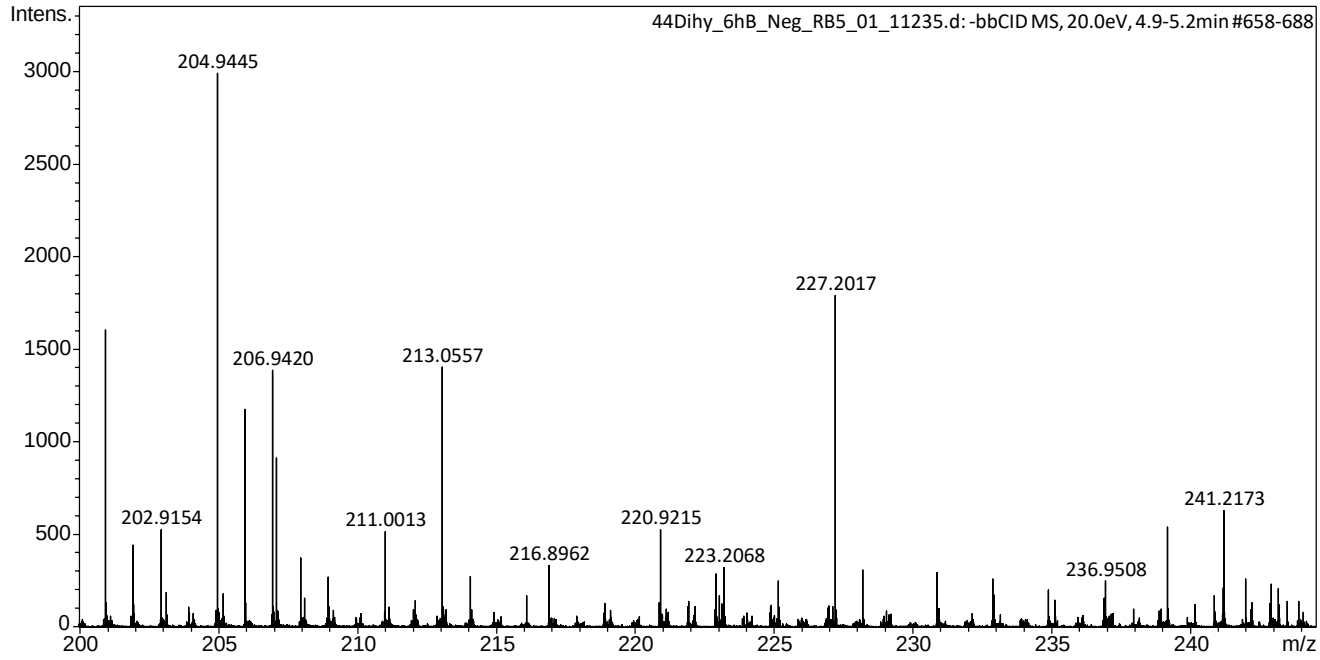
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

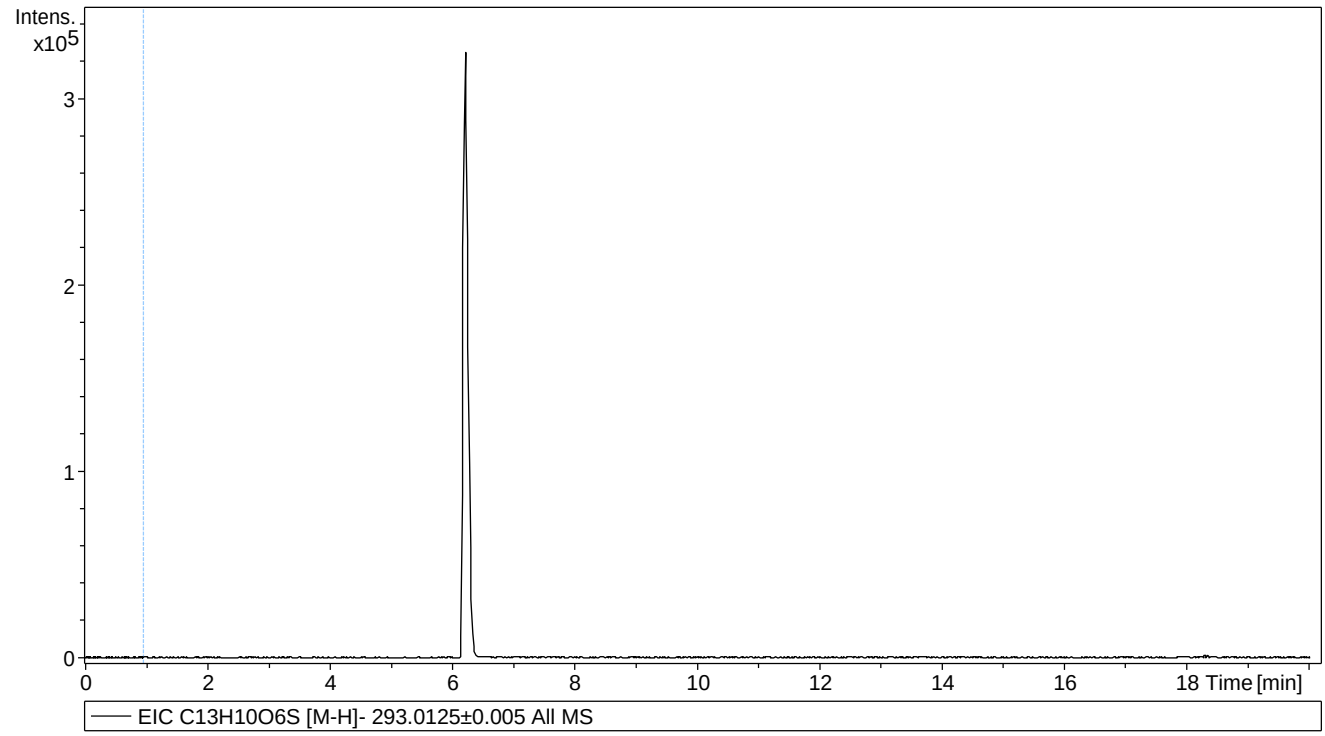
Operator BDAL@DE

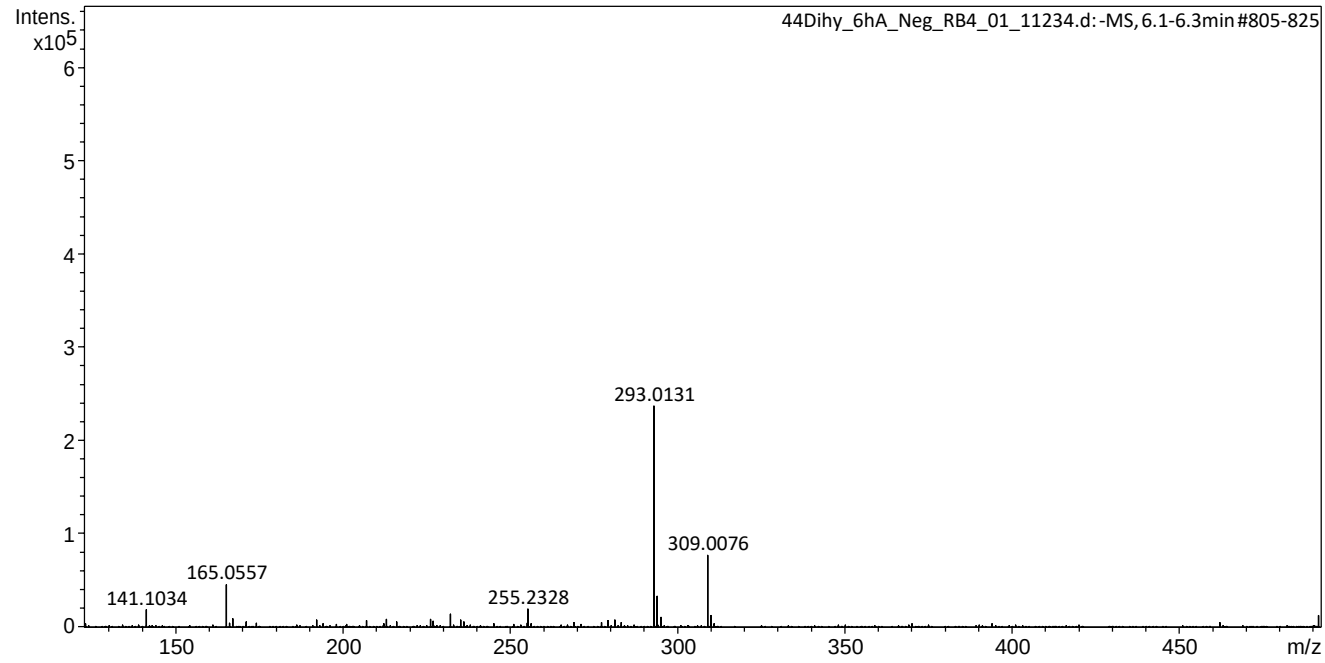
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

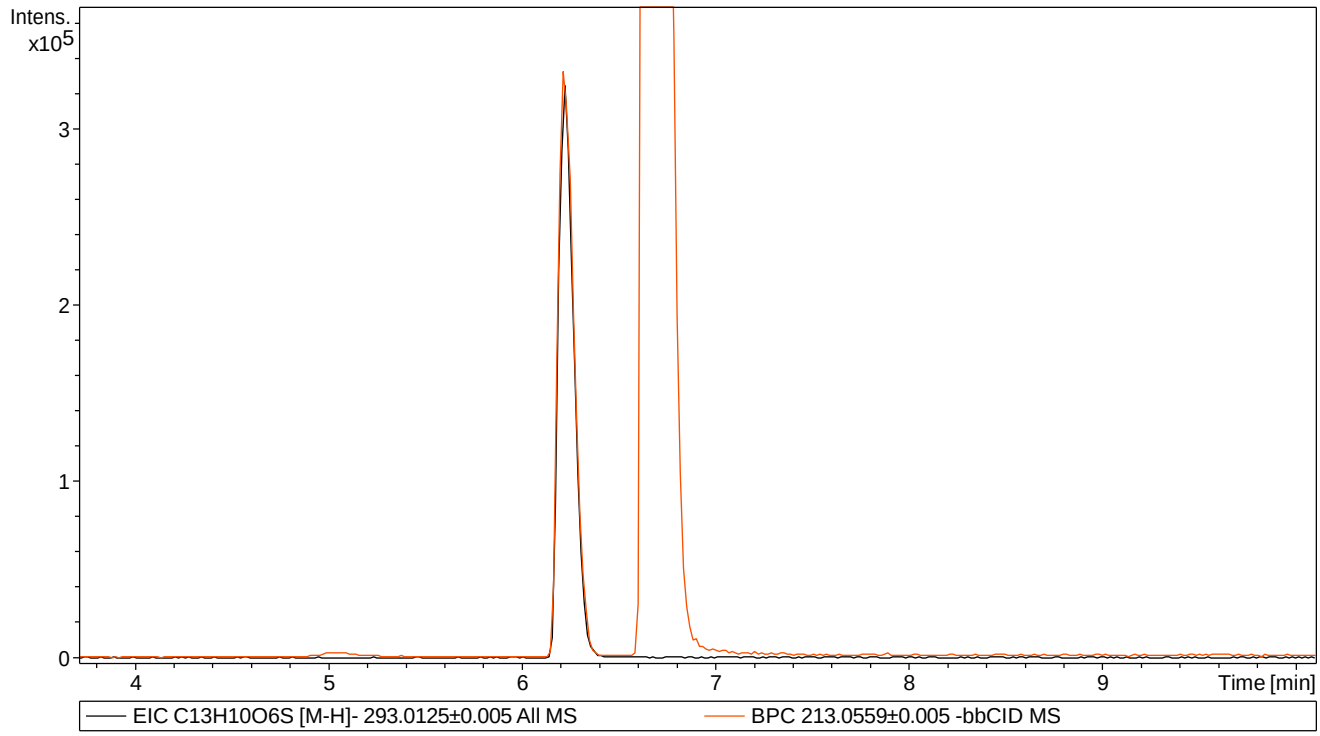
Operator BDAL@DE

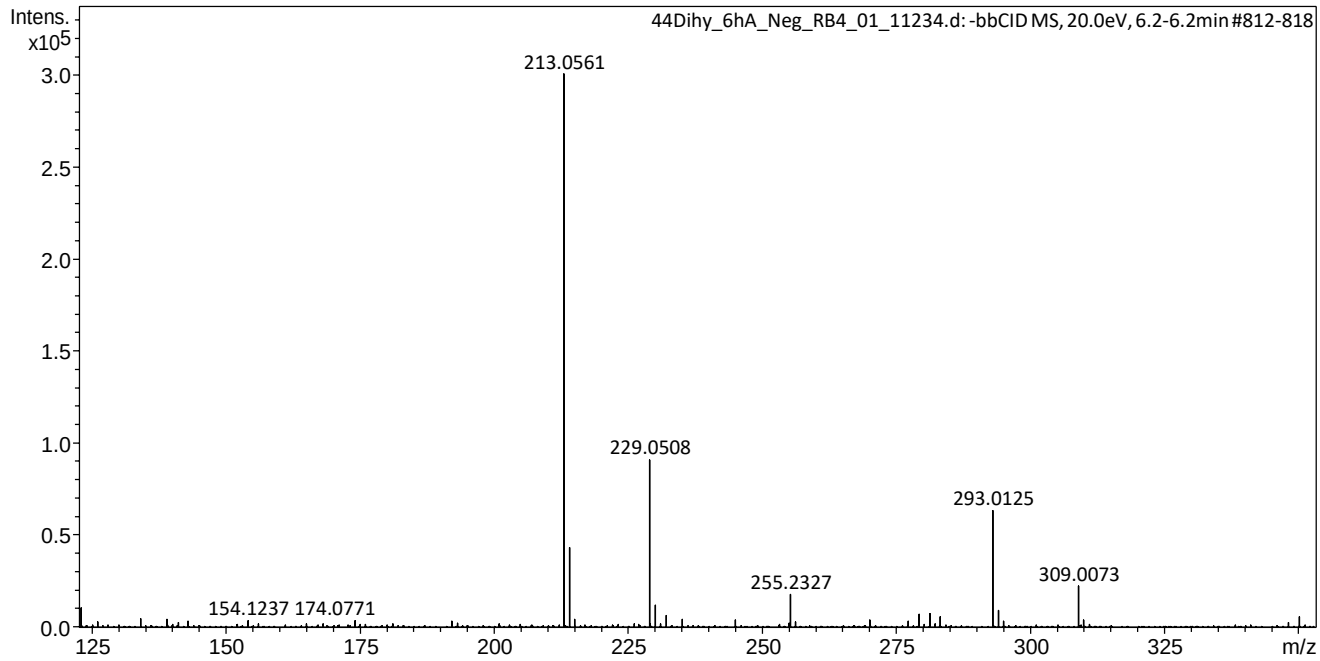
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

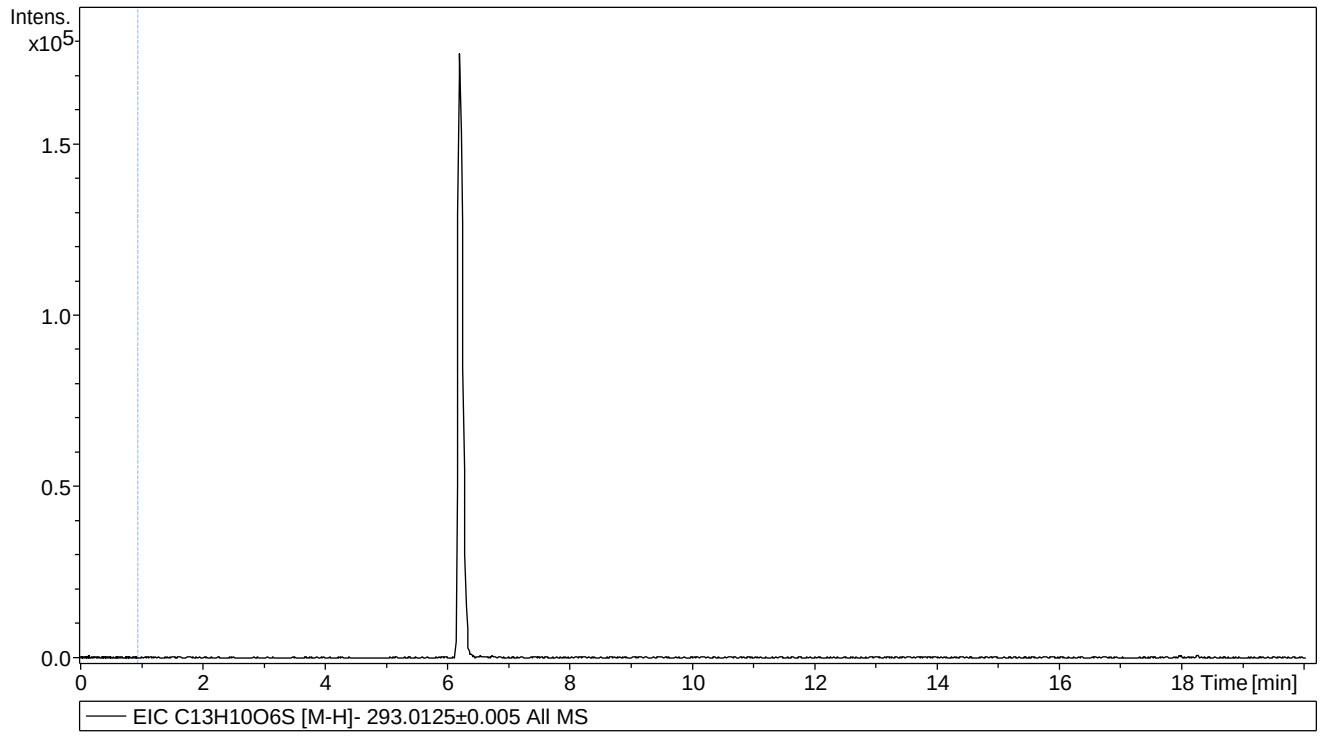
Operator BDAL@DE

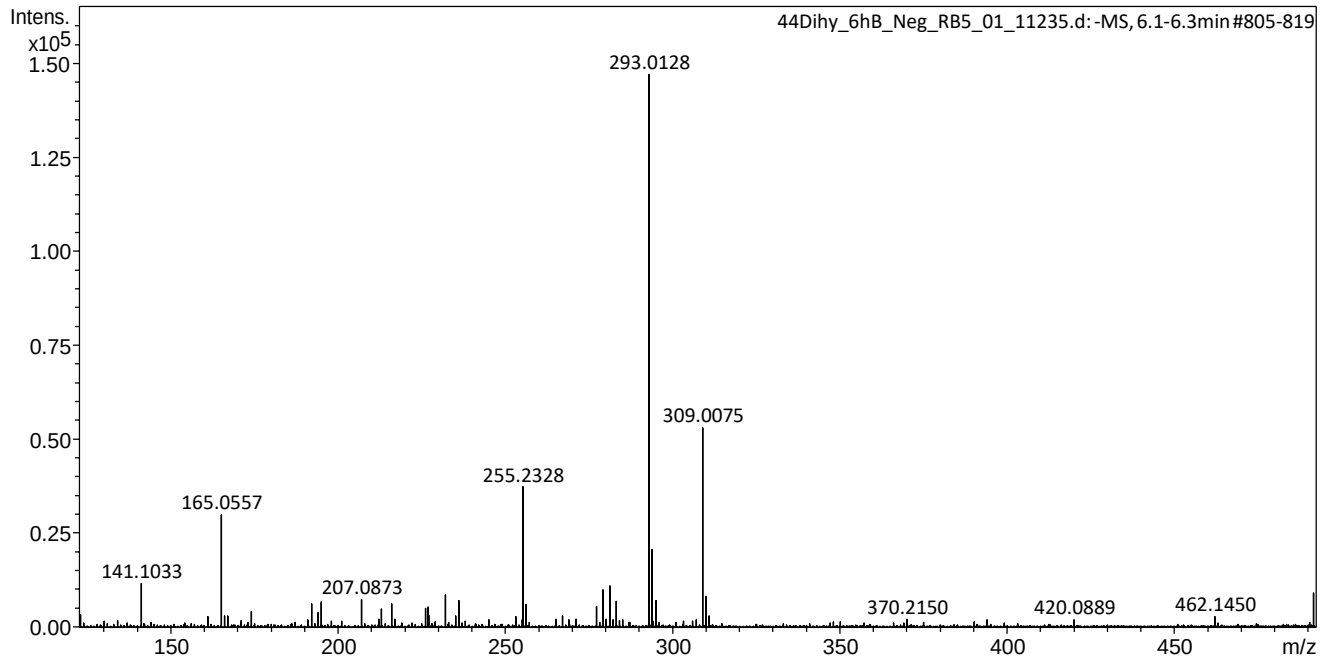
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

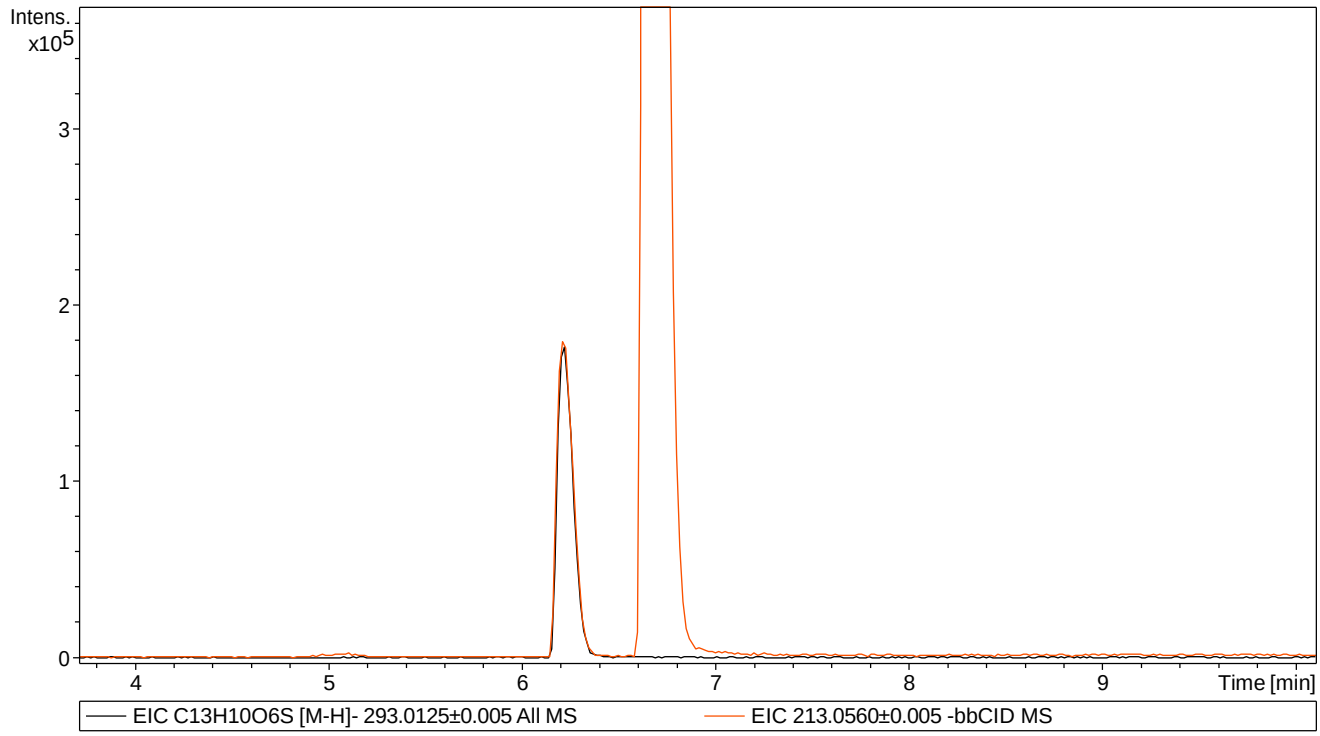
Operator BDAL@DE

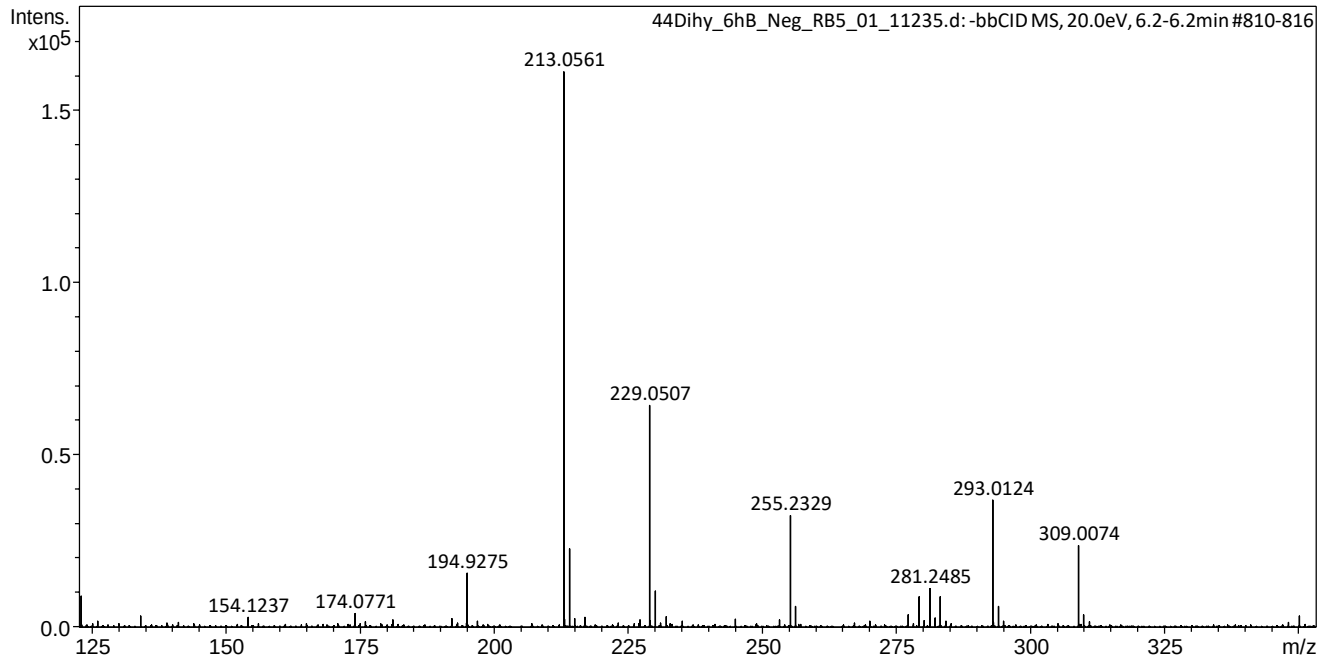
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:40:08

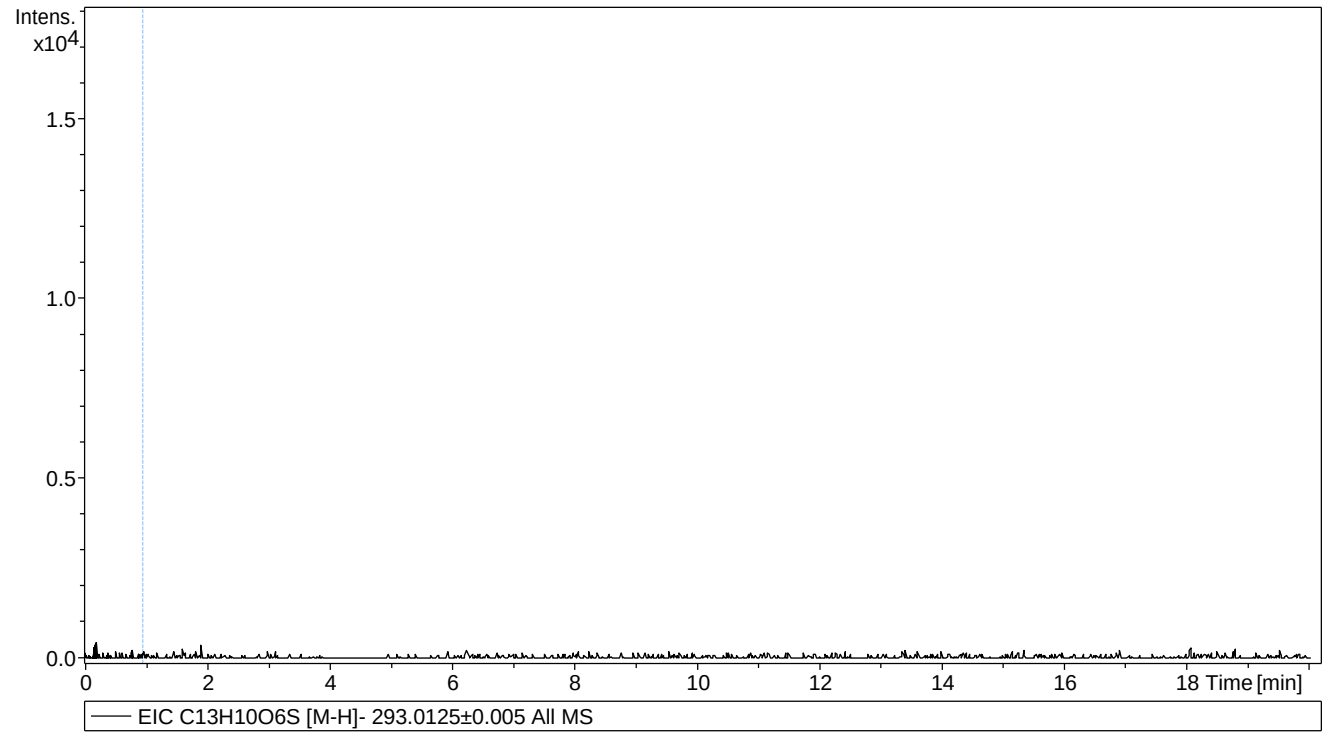
Operator BDAL@DE

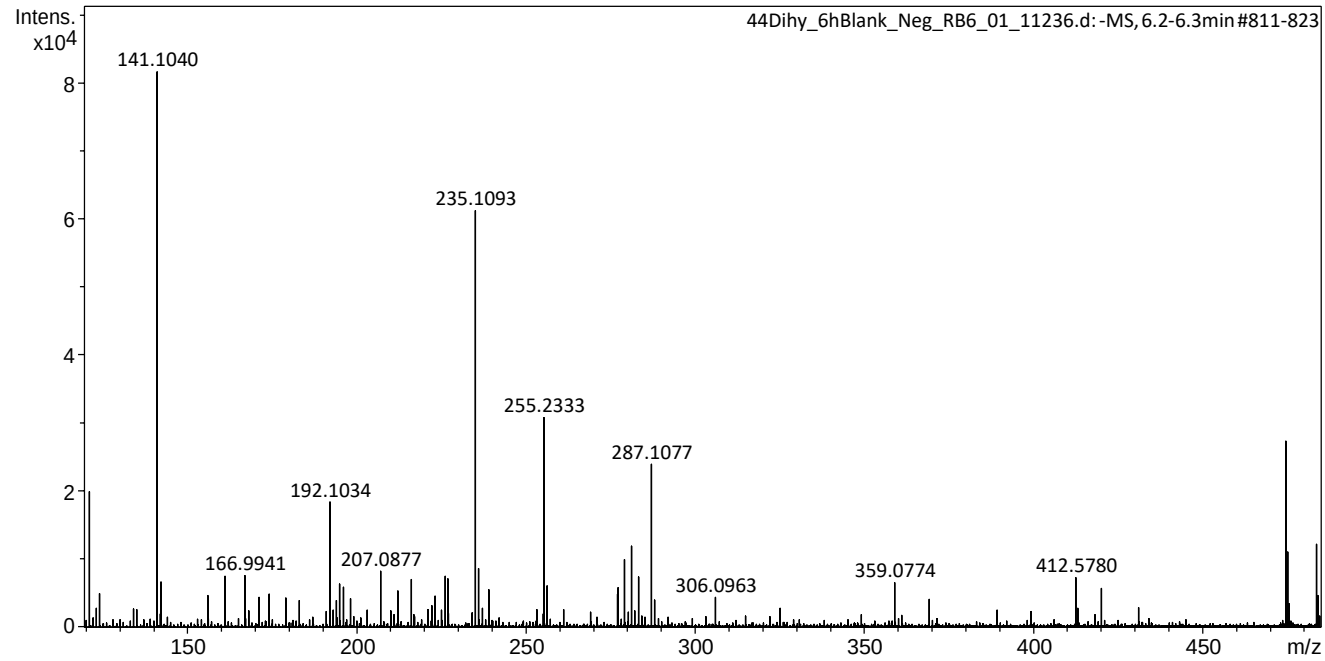
Sample Name 44Dihy_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

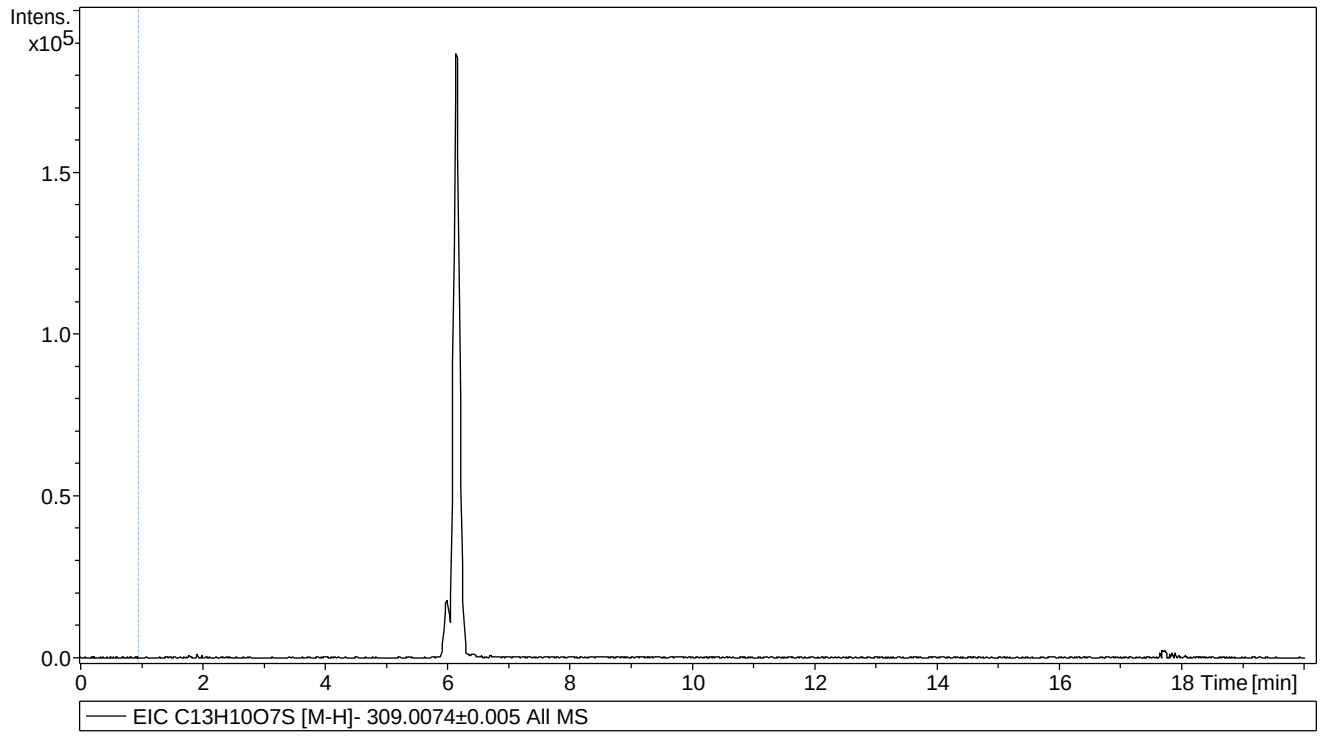
Operator BDAL@DE

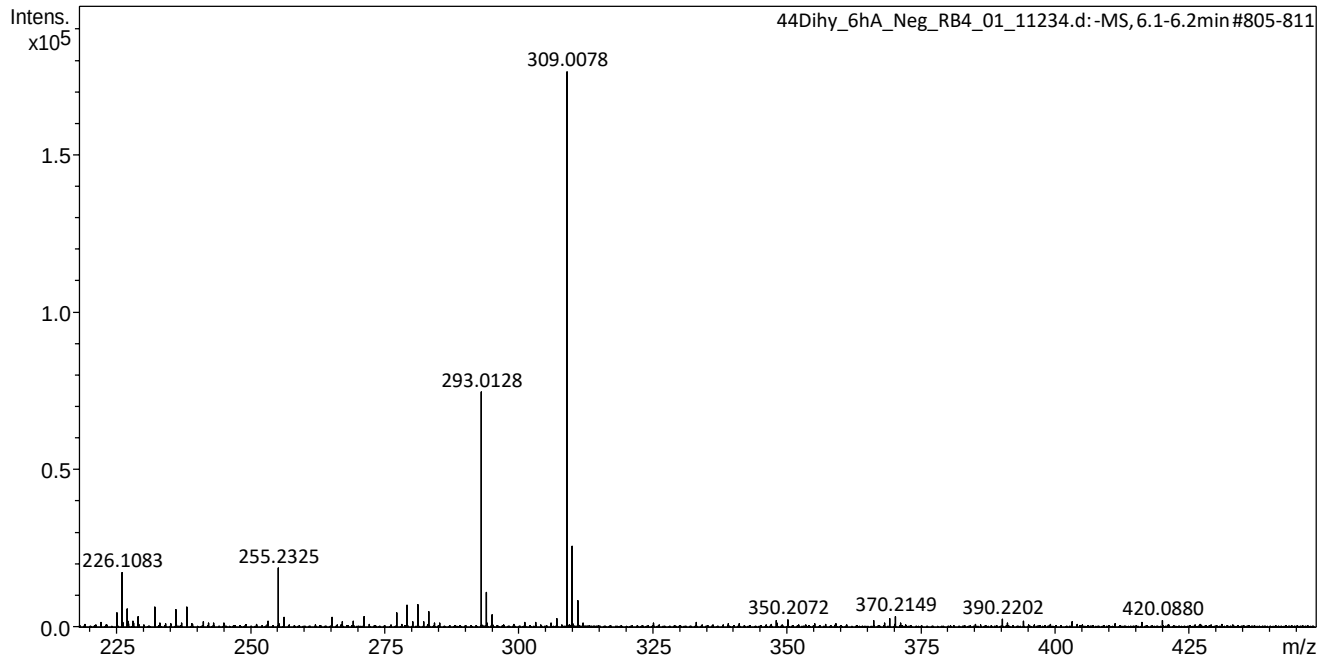
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 00:57:38

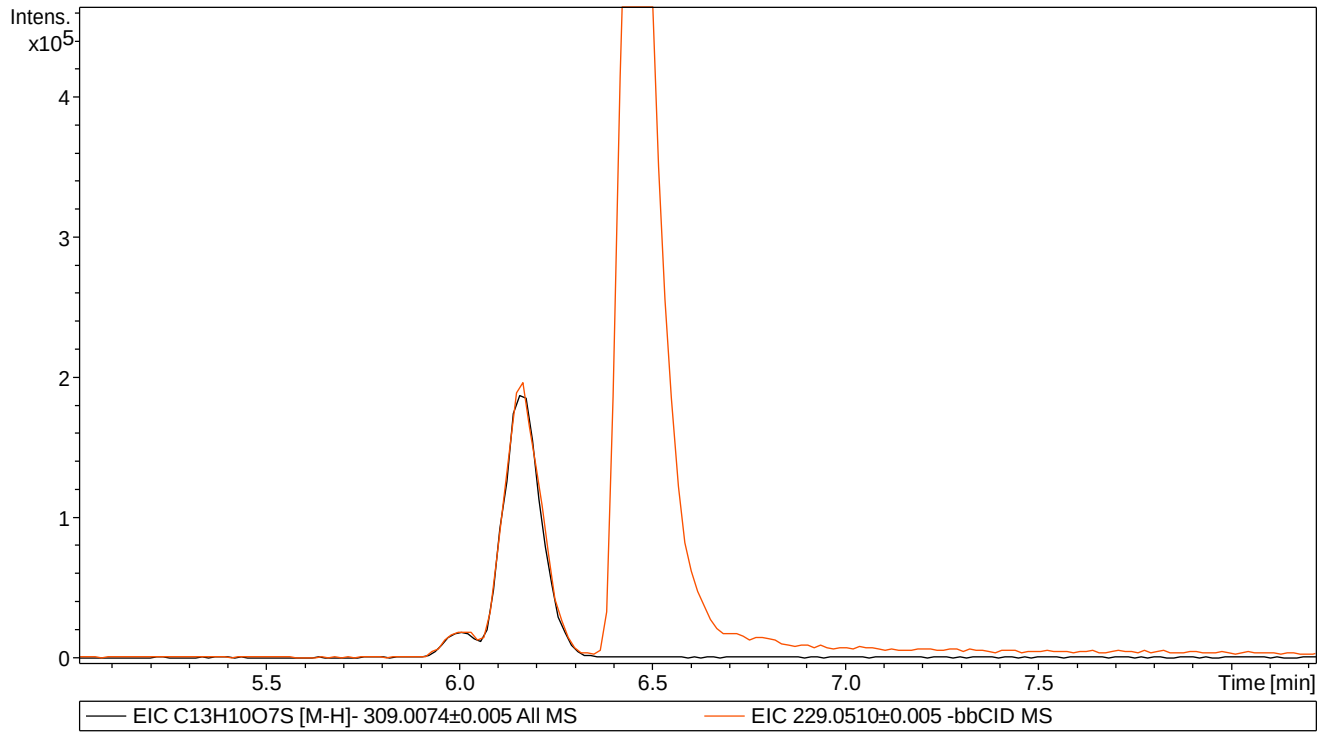
Operator BDAL@DE

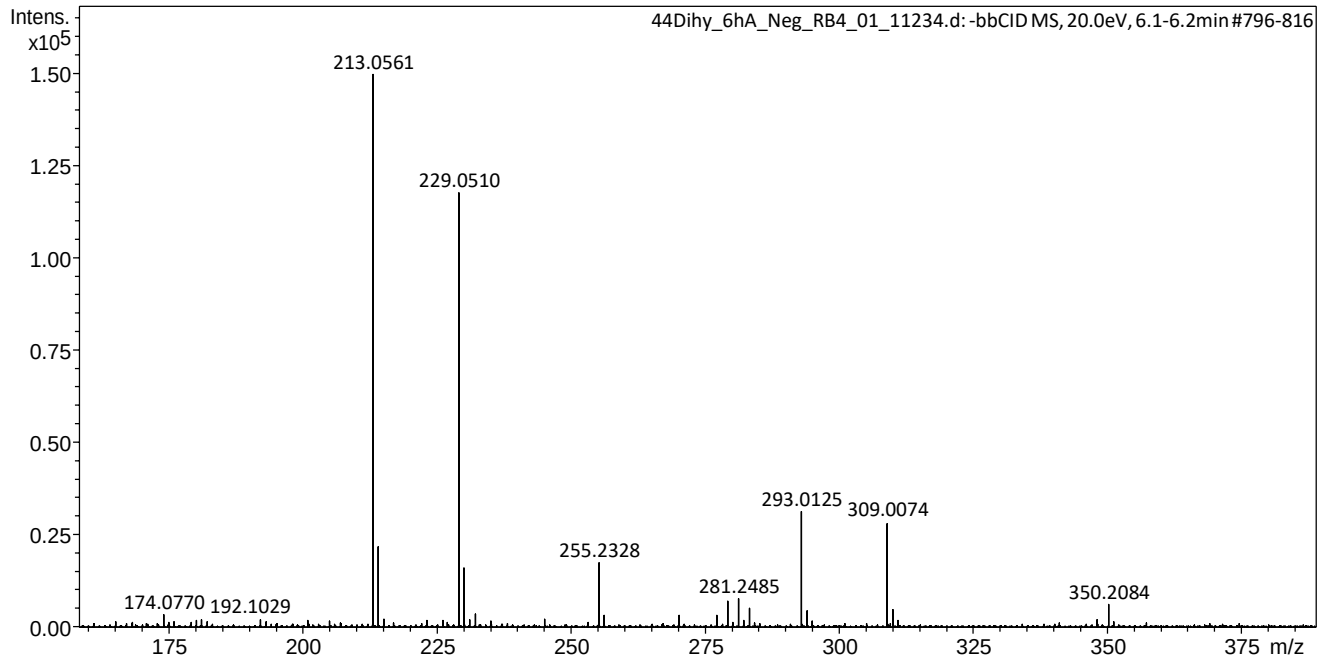
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

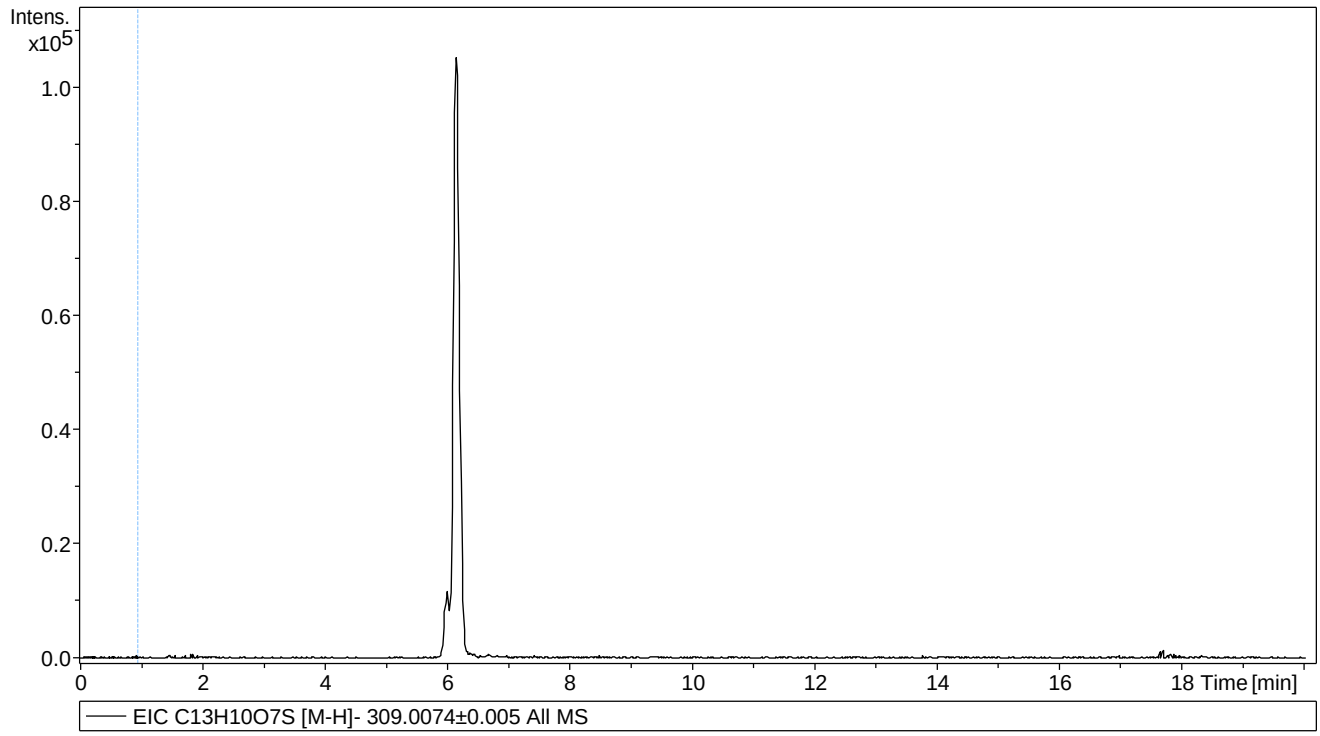
Operator BDAL@DE

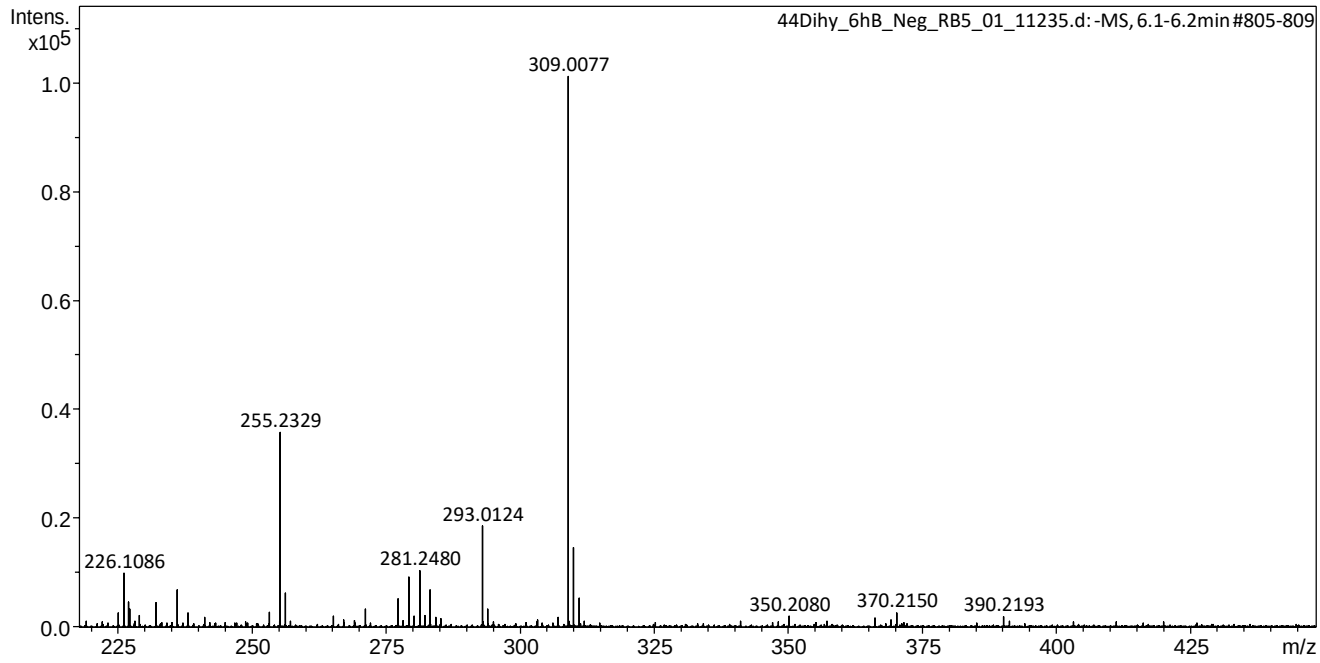
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:18:53

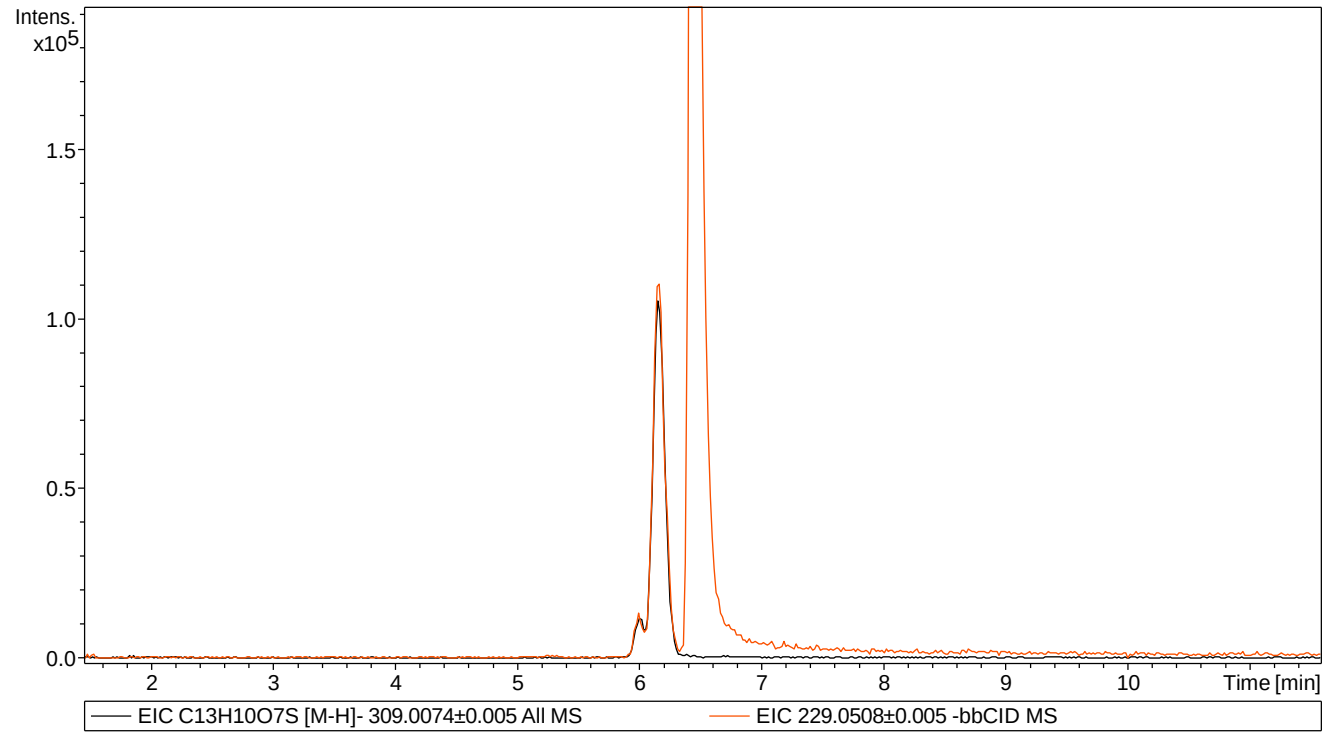
Operator BDAL@DE

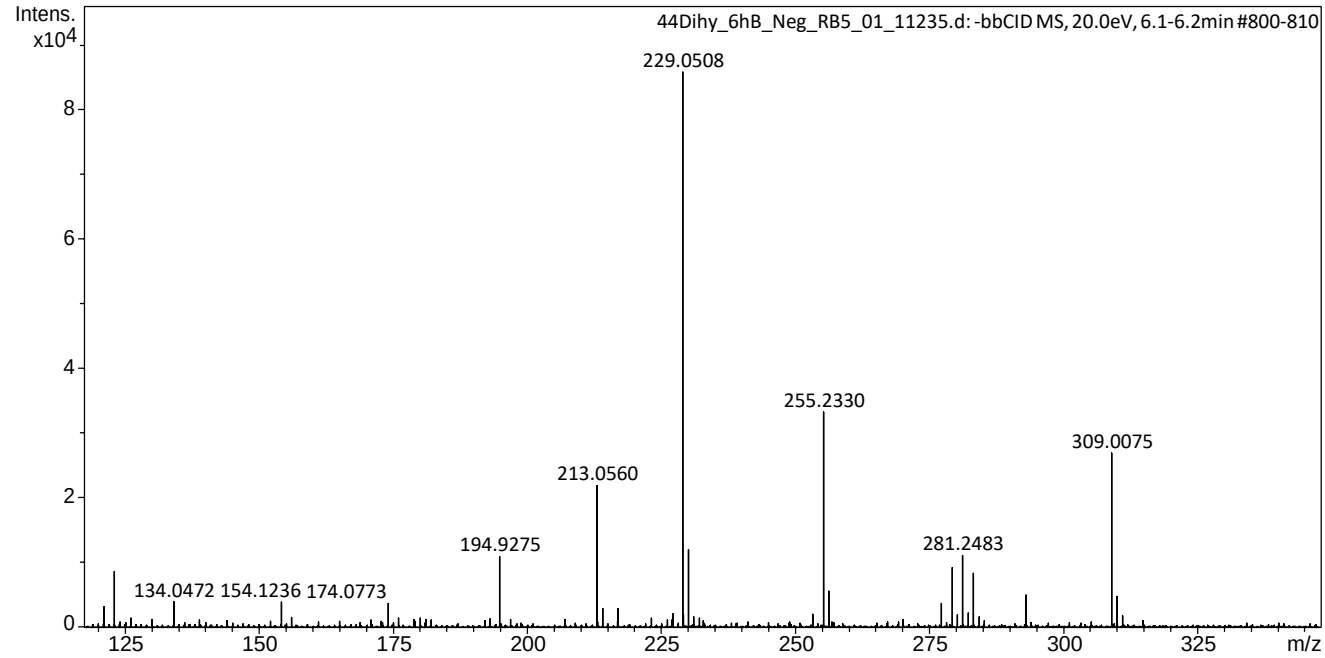
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:40:08

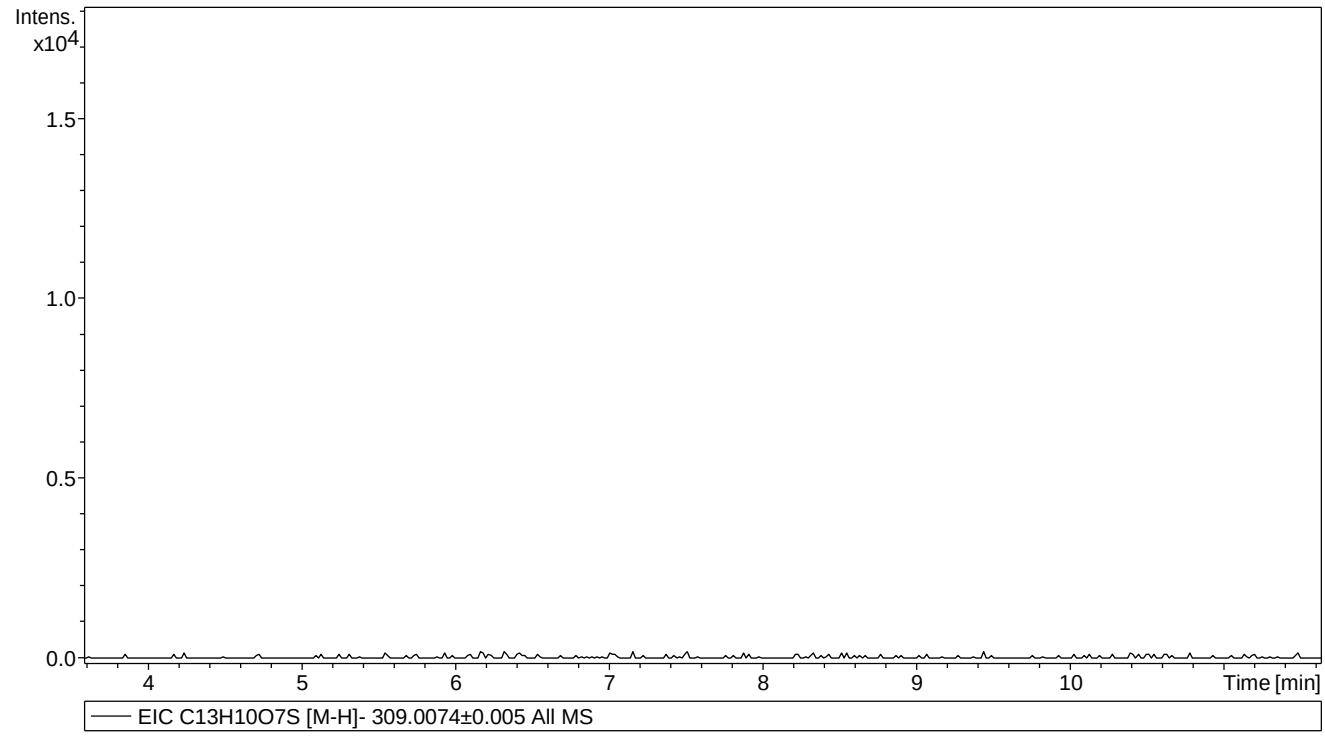
Operator BDAL@DE

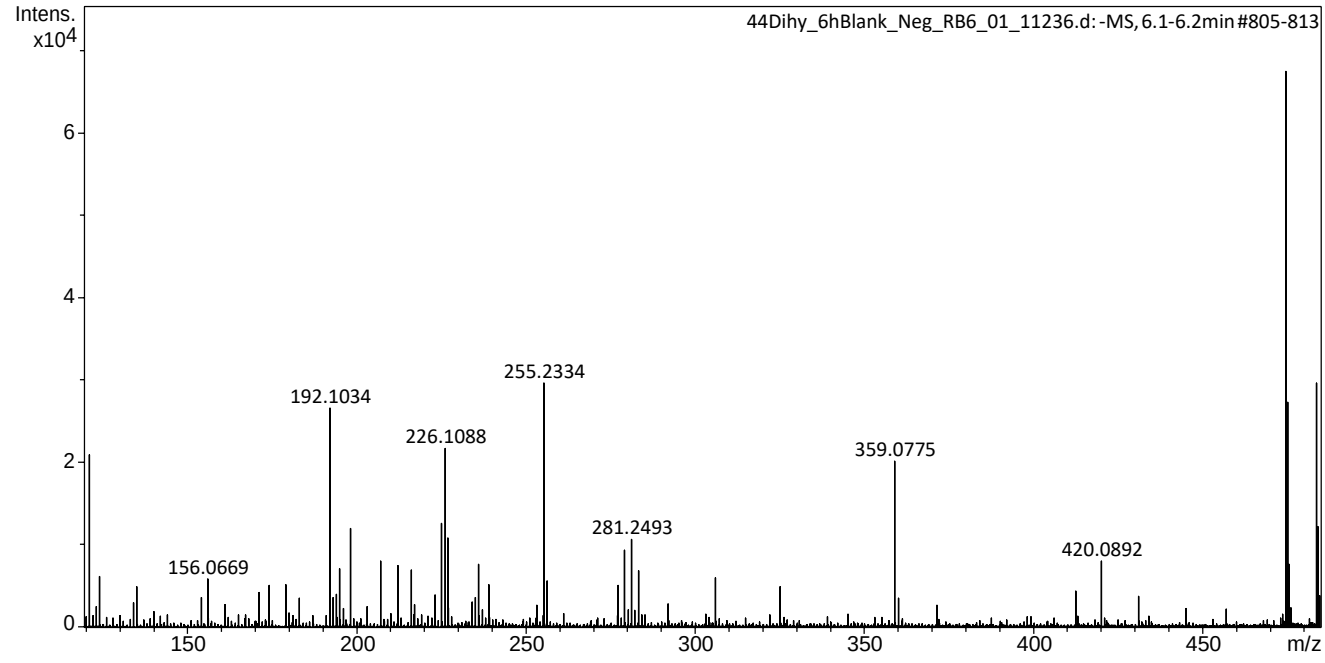
Sample Name 44Dihy_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/201700 :57:38

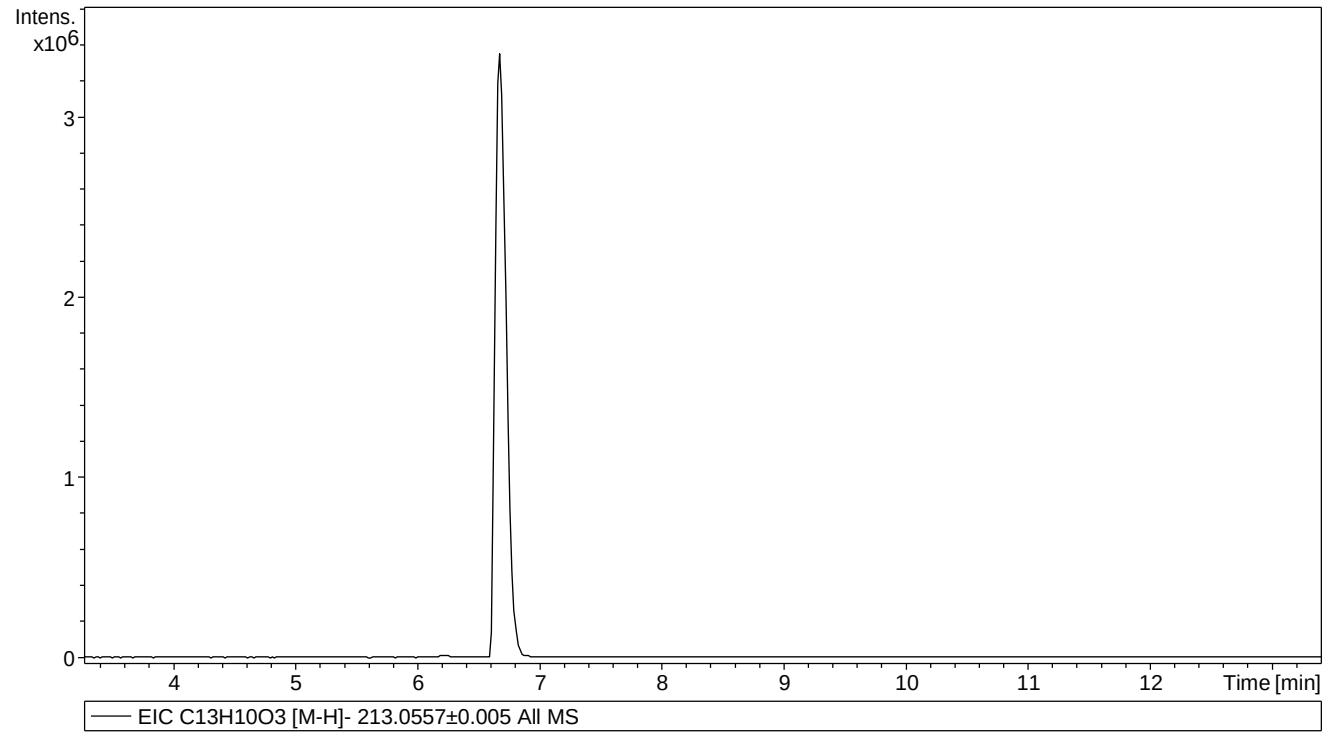
Operator BDAL@DE

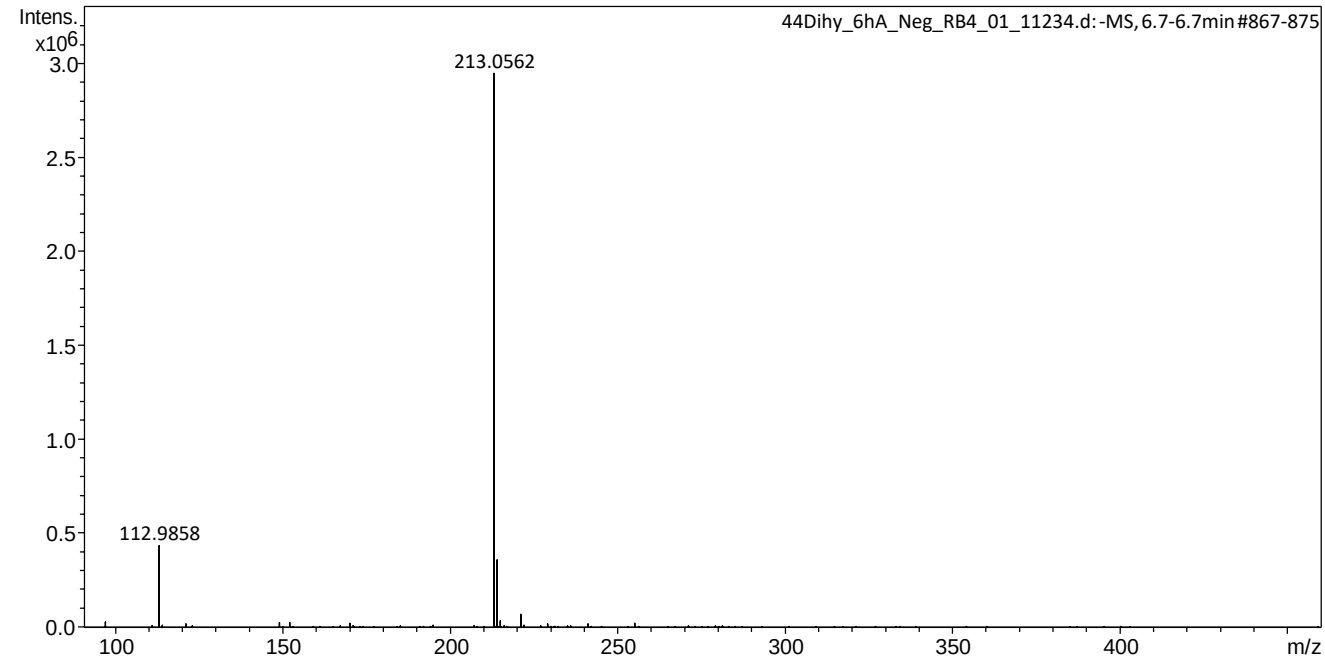
Sample Name 44Dihy_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/201701 :18:53

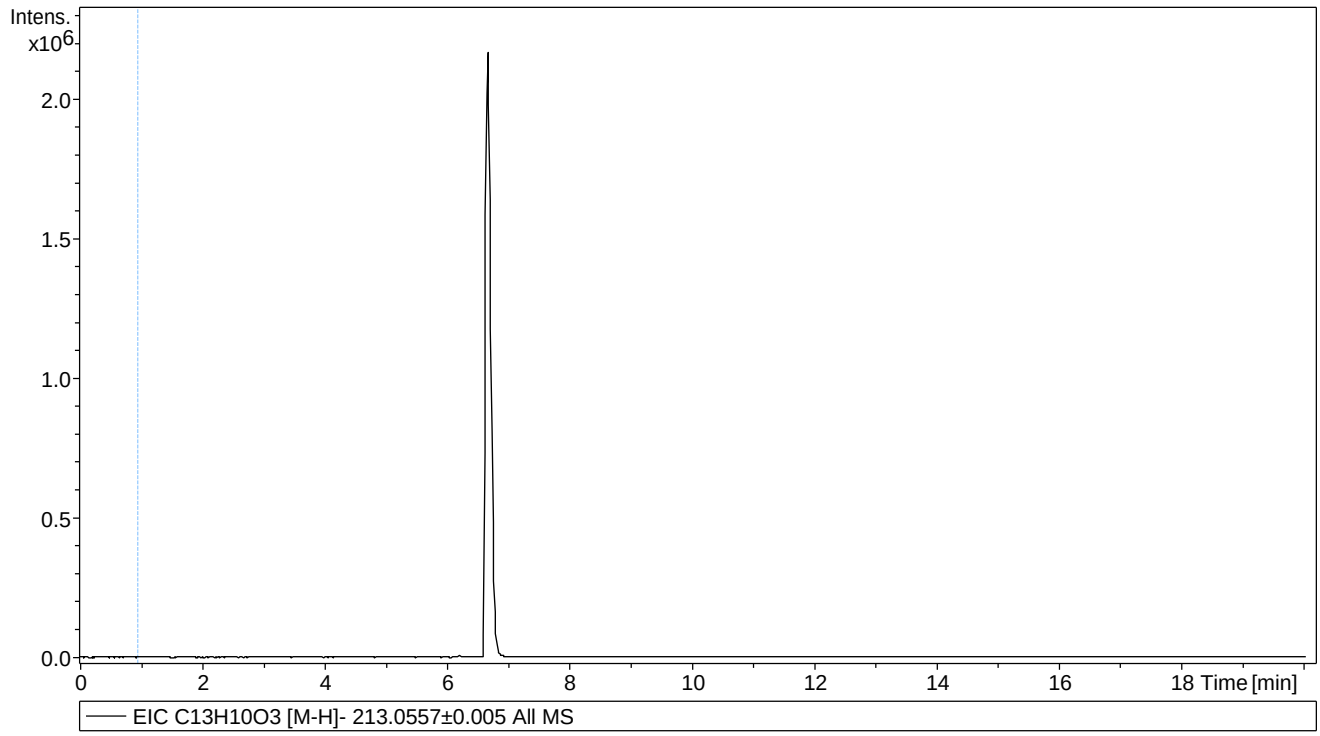
Operator BDAL@DE

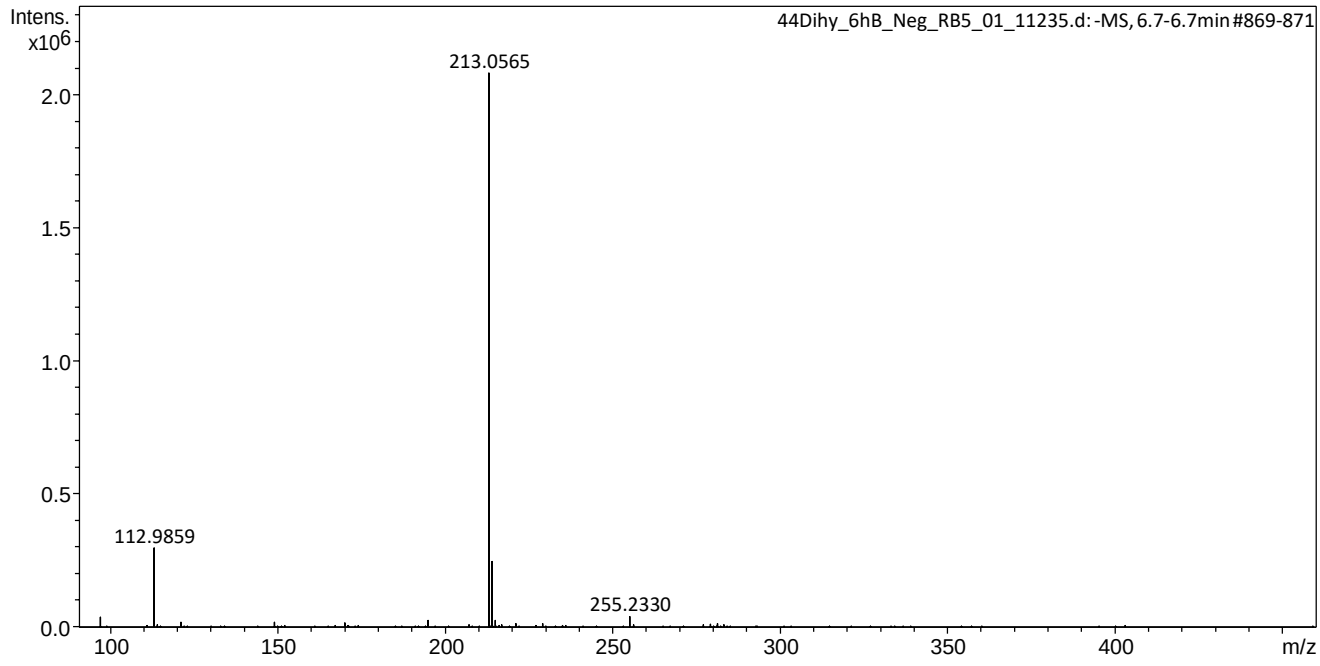
Sample Name 44Dihy_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 01:40:08

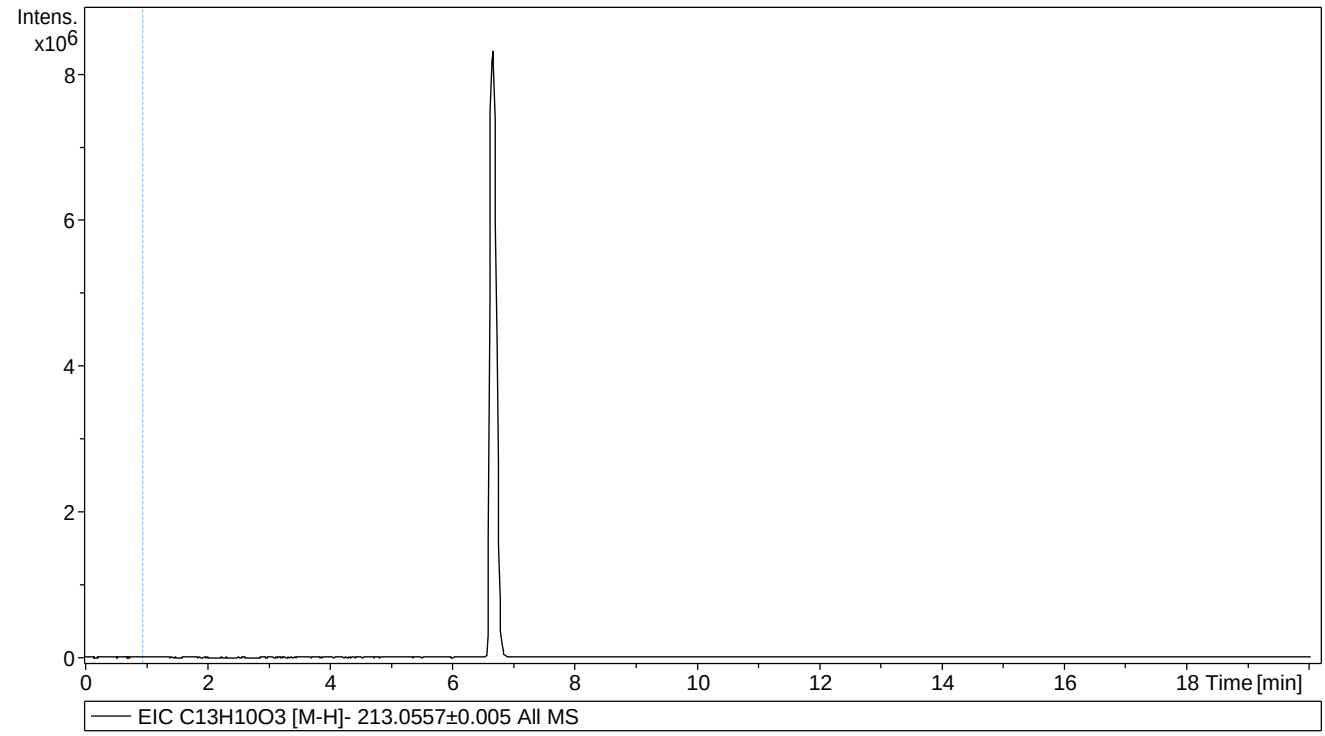
Operator BDAL@DE

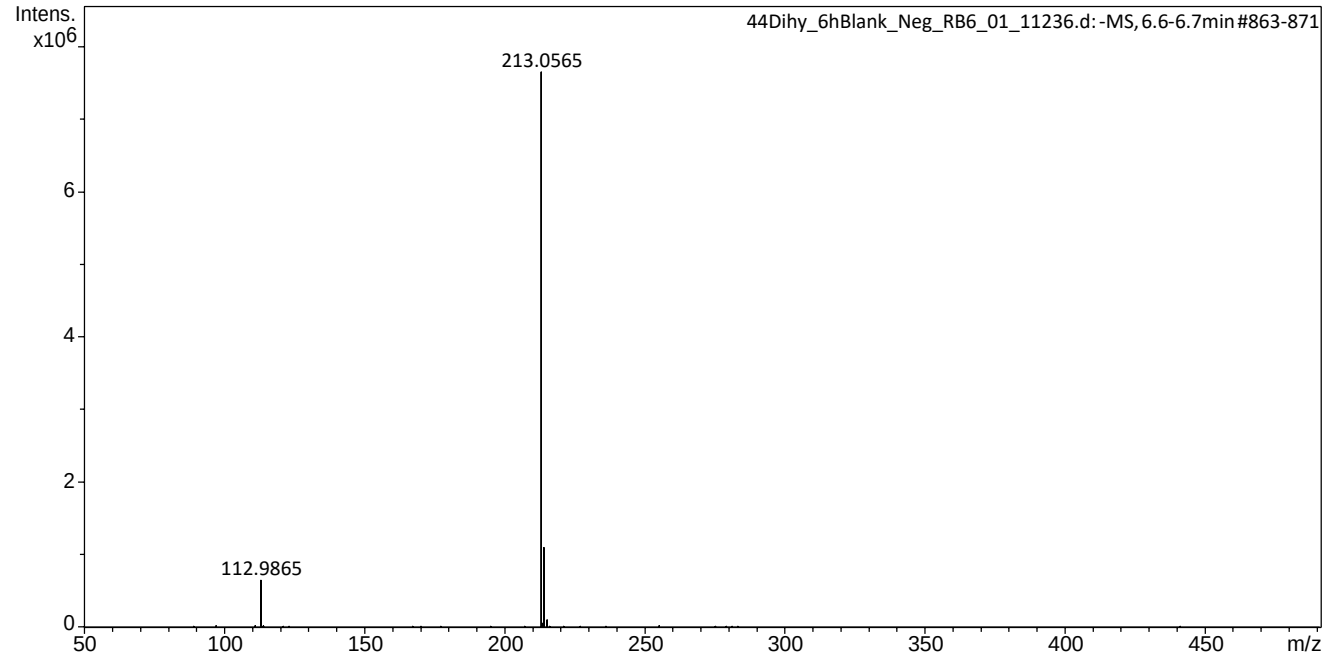
Sample Name 44Dihy_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

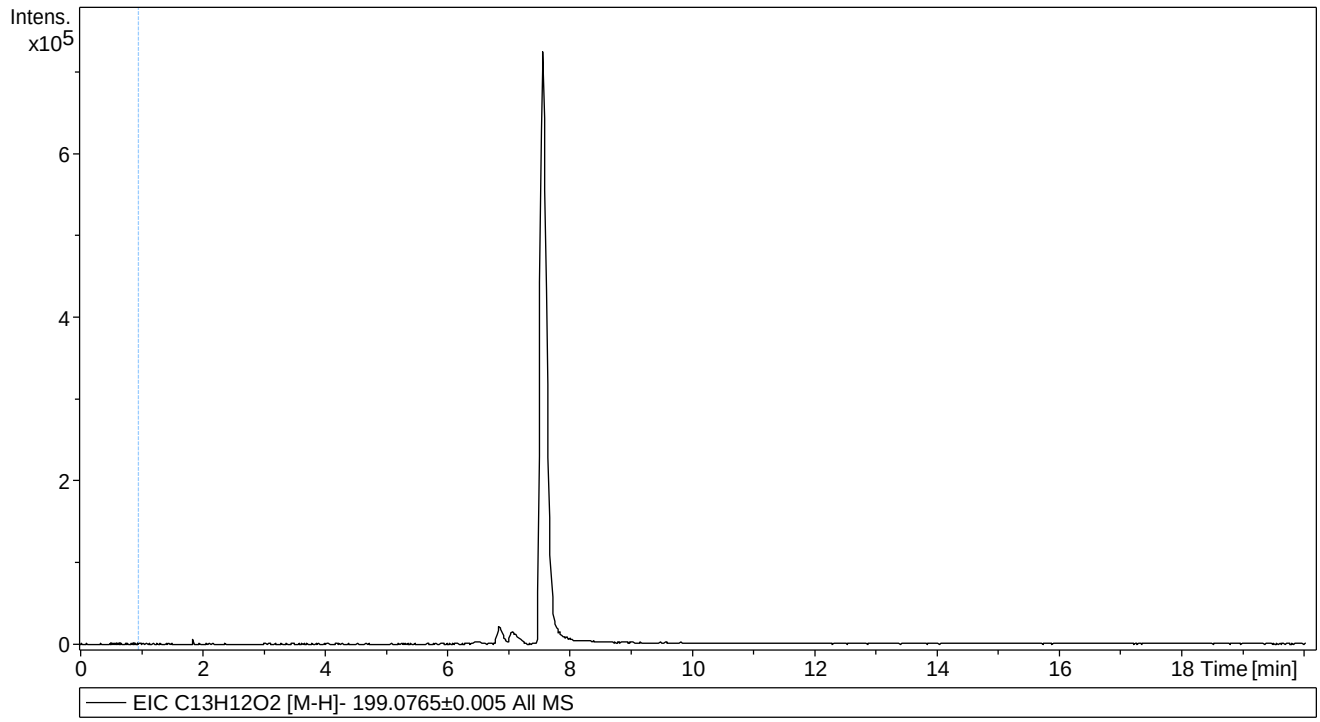
Operator BDAL@DE

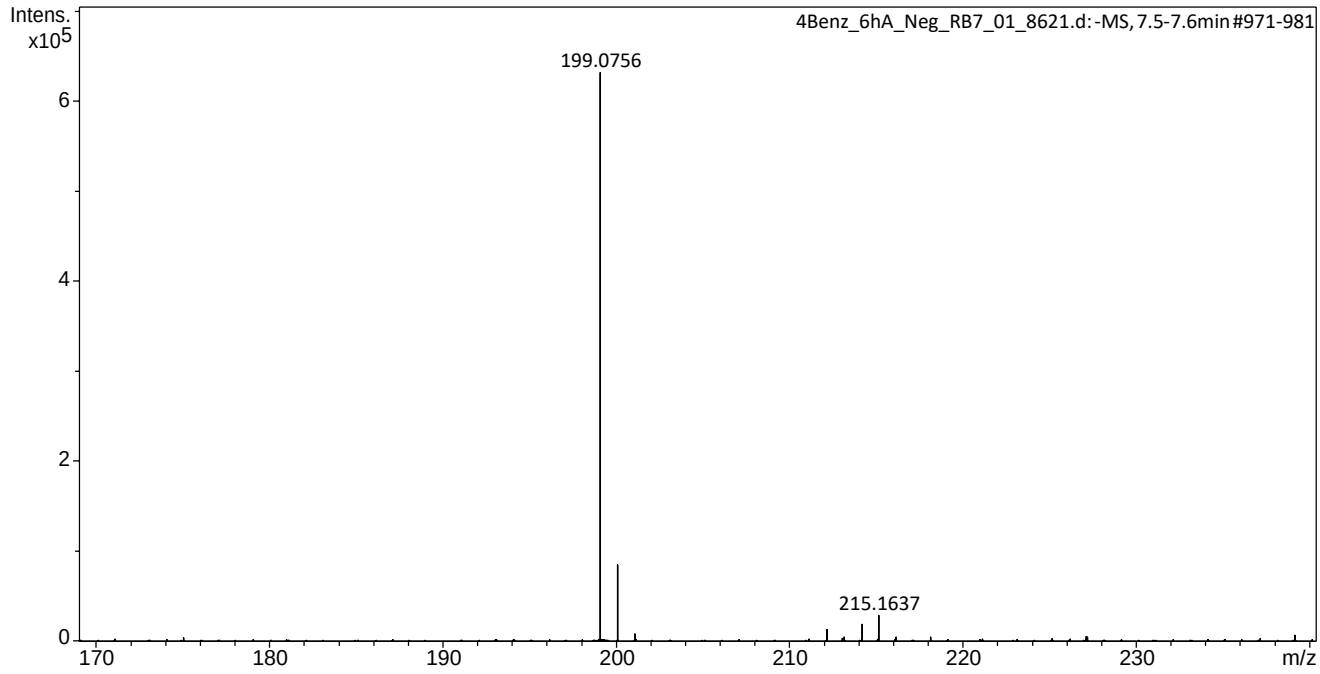
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

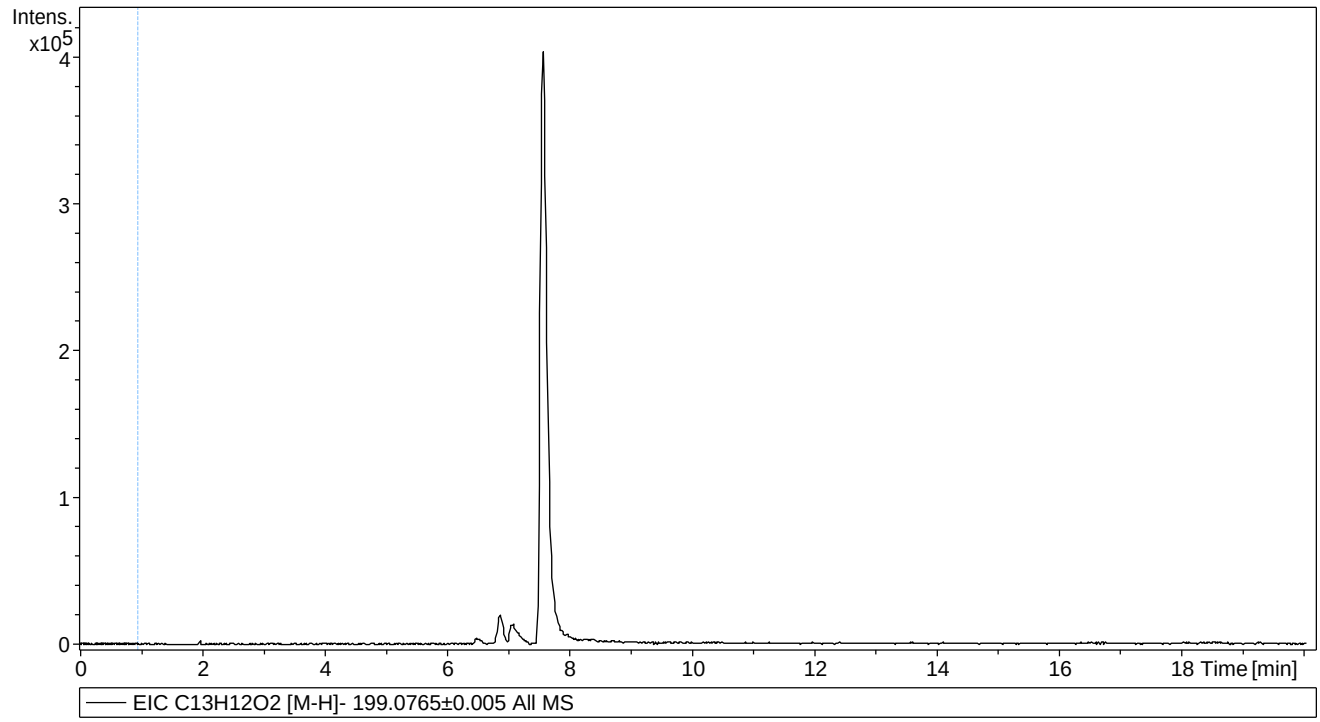
Operator BDAL@DE

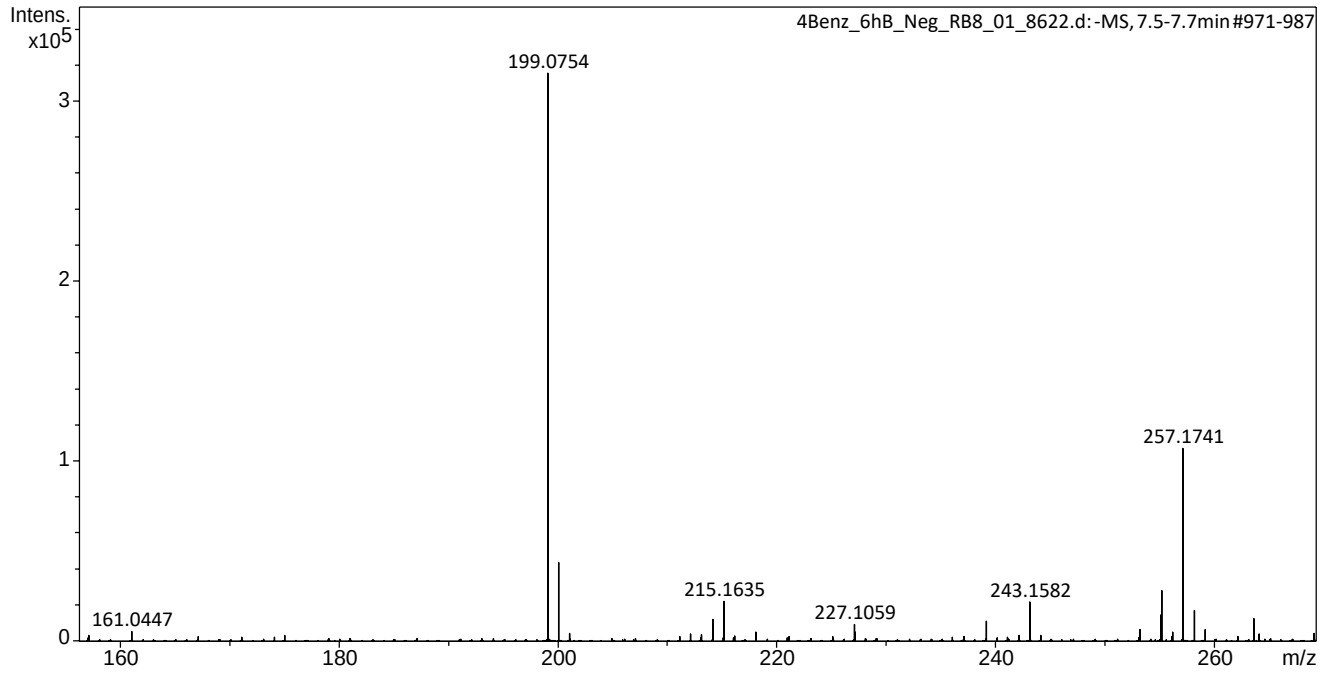
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





4Benz_6hB_Neg_RB8_01_8622.d

Display Report

Analysis Info

Acquisition Date 07/08/2017 23:54:18

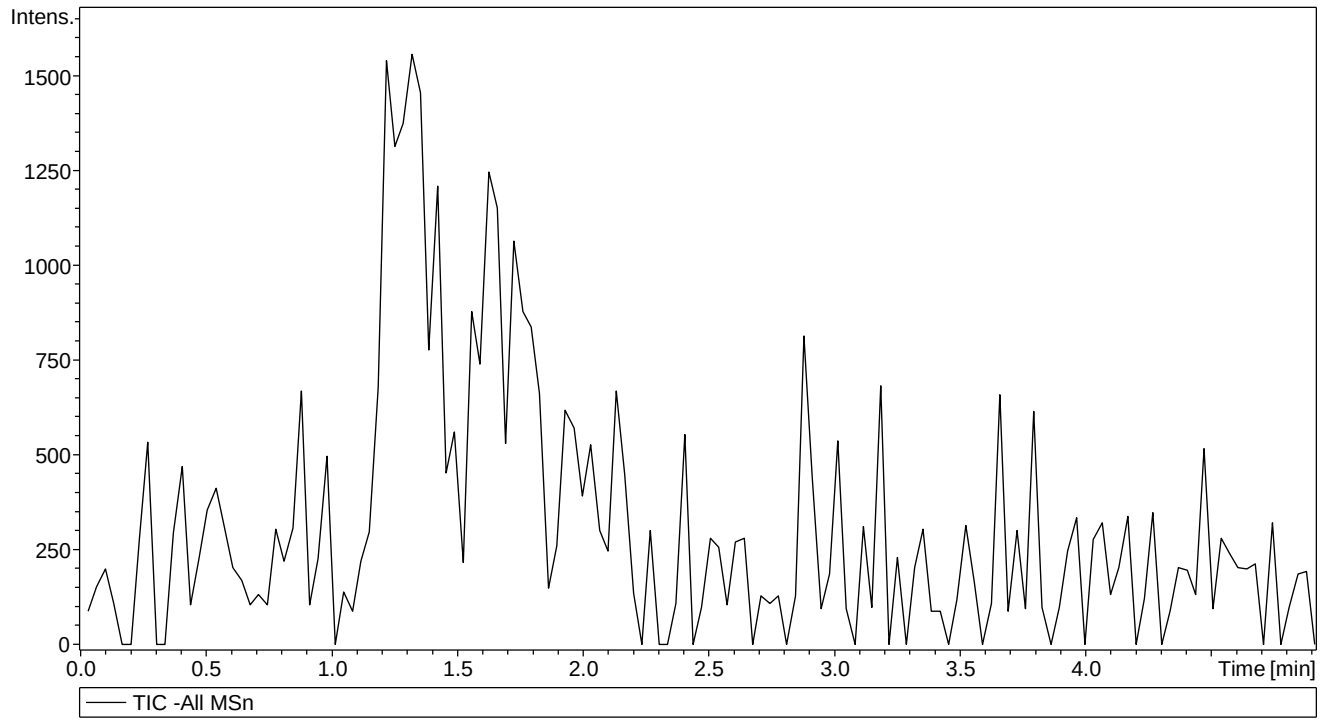
Operator BDAL@DE

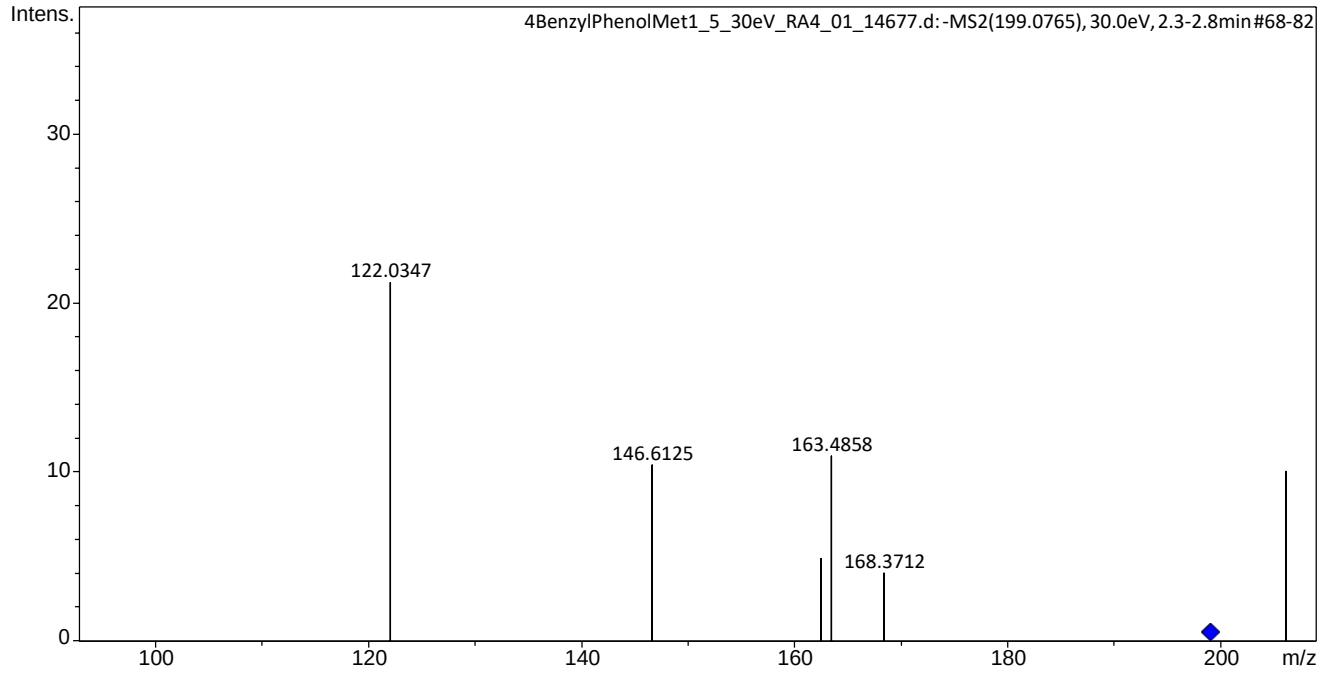
Sample Name 4BenzylPhenolMet1_5_30eV

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	75 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





4BenzylPhenolMet1_5_30eV_RA4_01_14677.d

Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

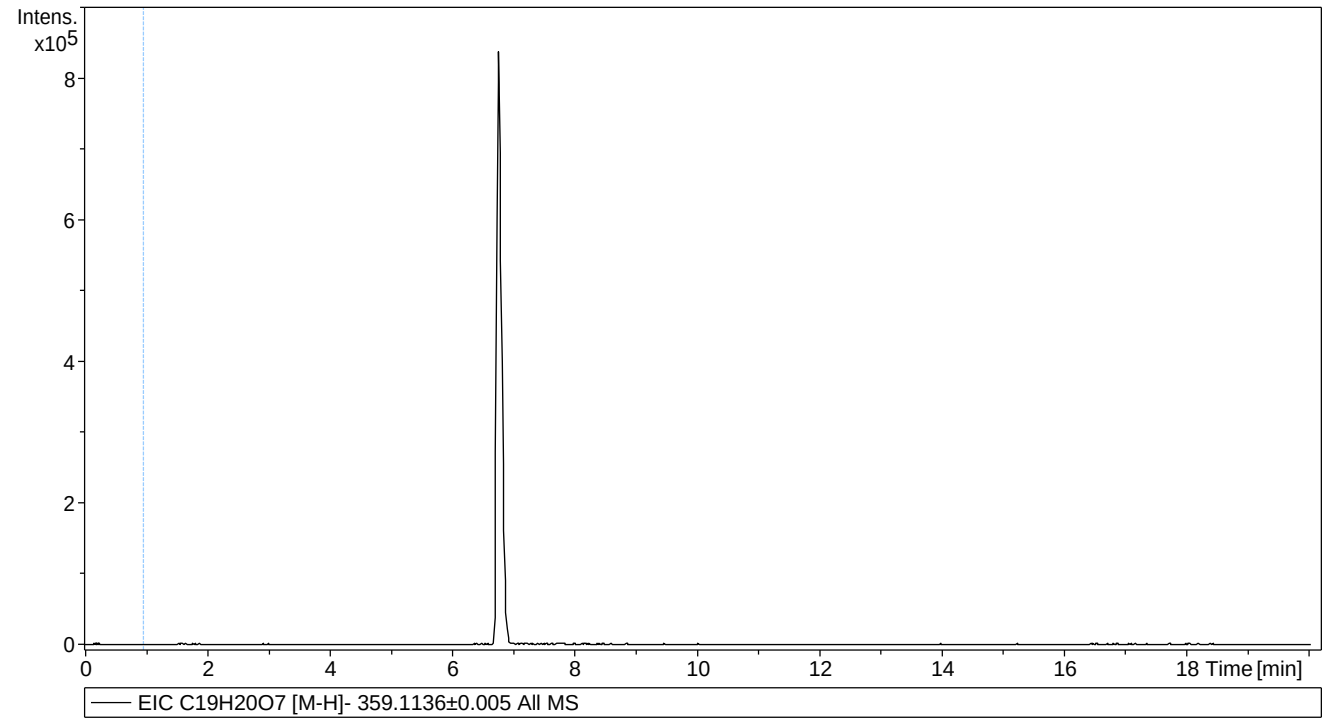
Operator BDAL@DE

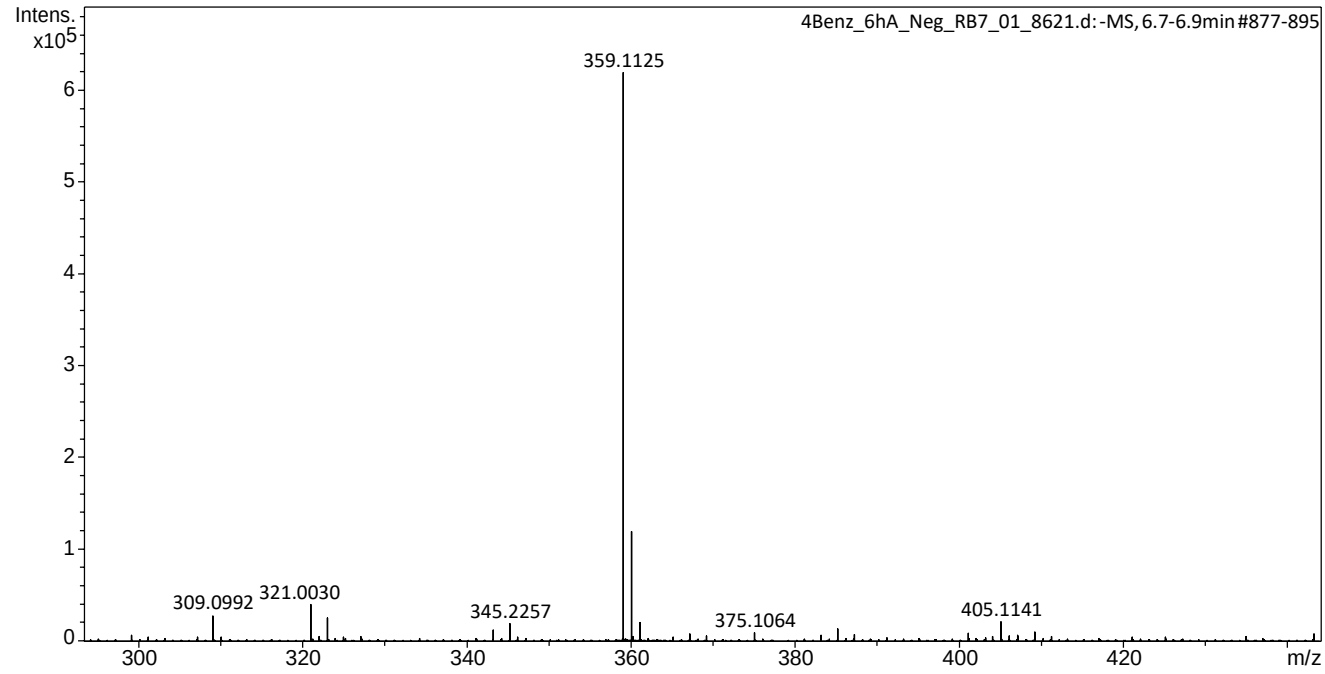
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





4Benz_6hA_Neg_RB7_01_8621.d

Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

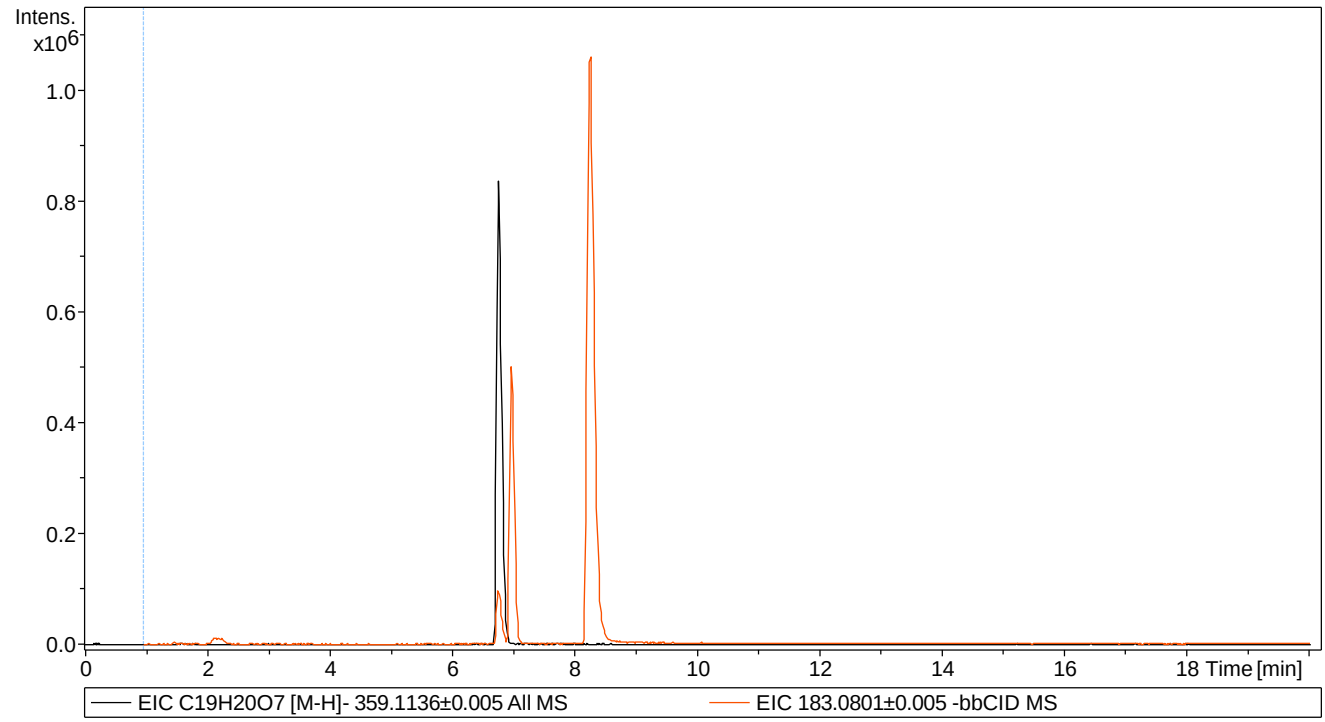
Operator BDAL@DE

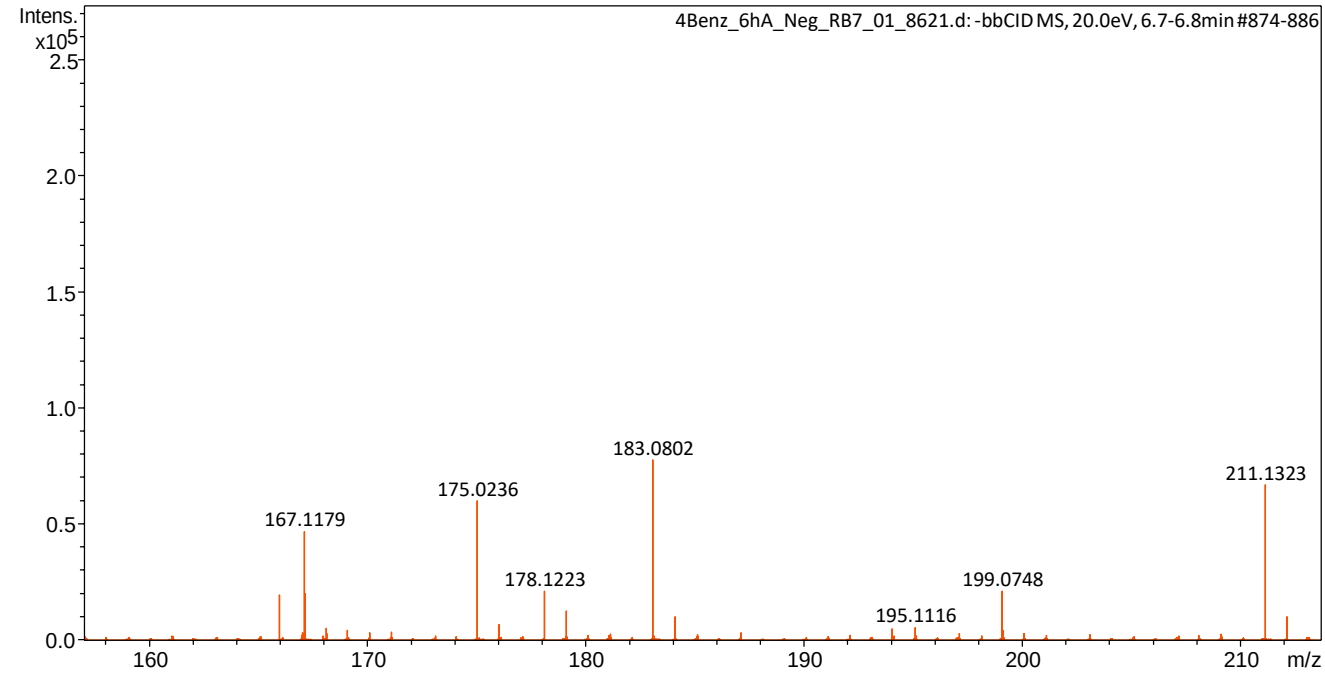
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

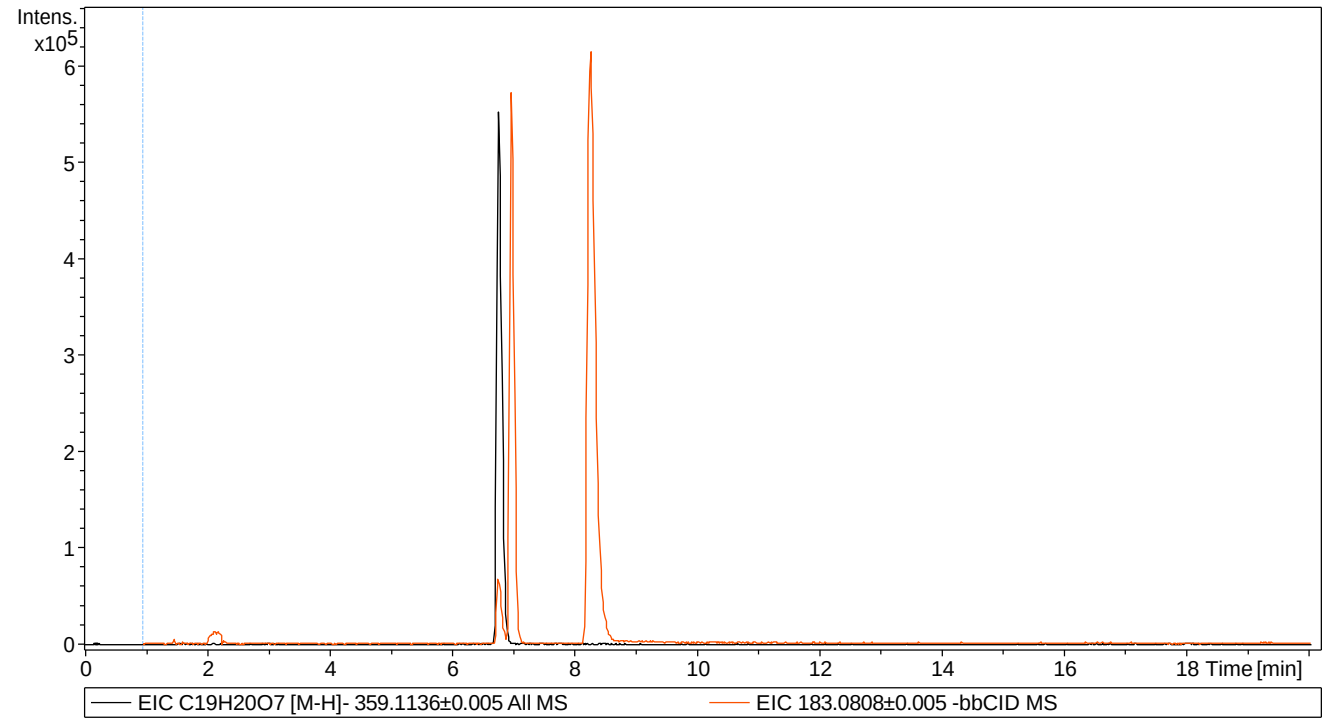
Operator BDAL@DE

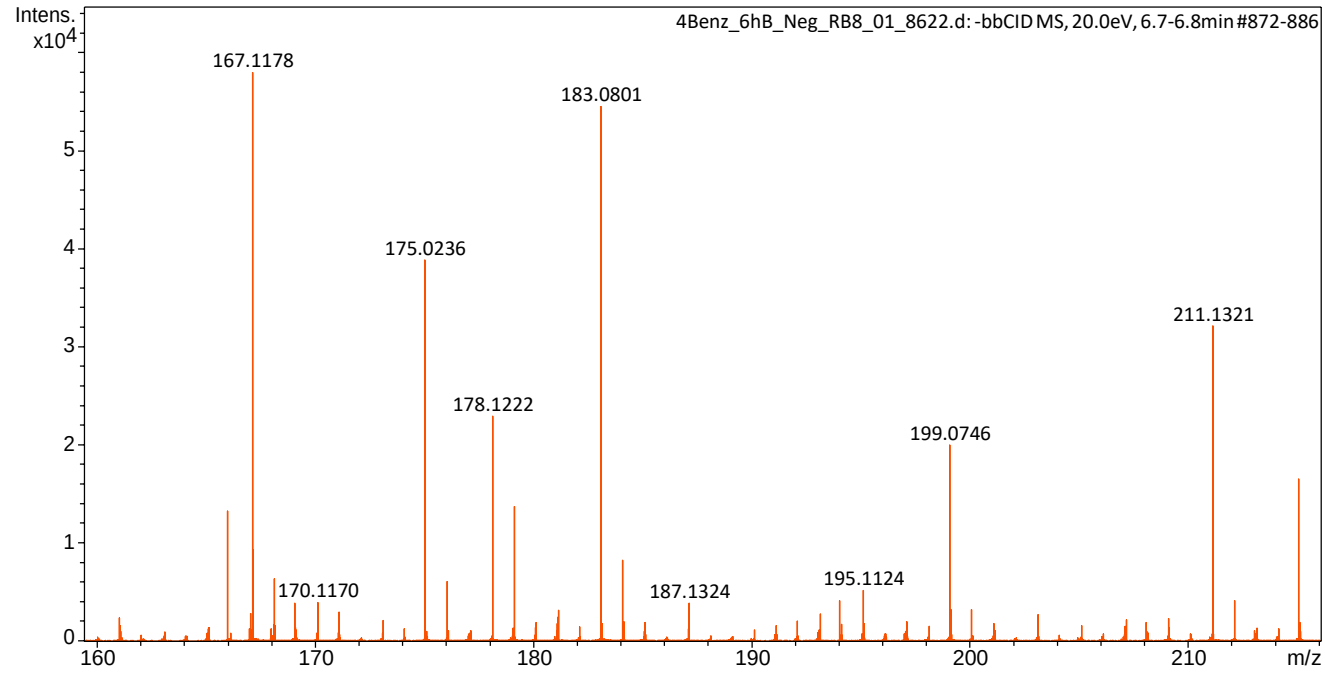
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

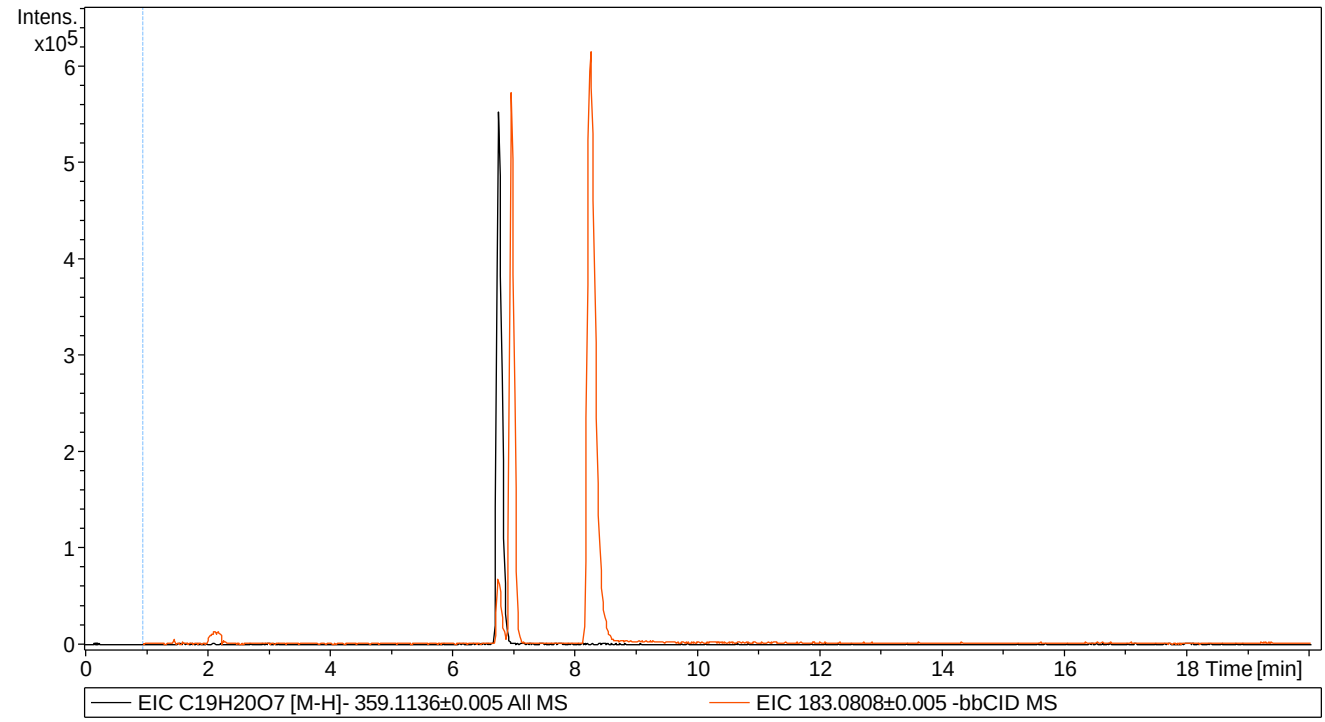
Operator BDAL@DE

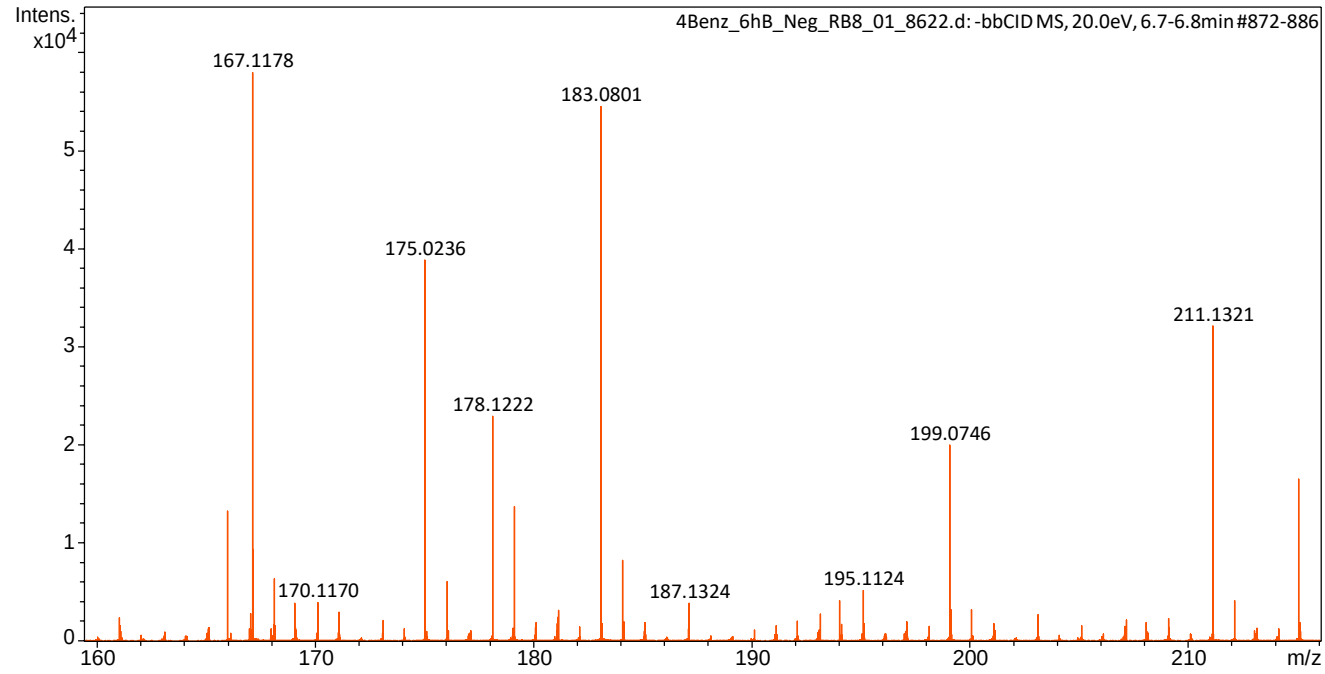
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

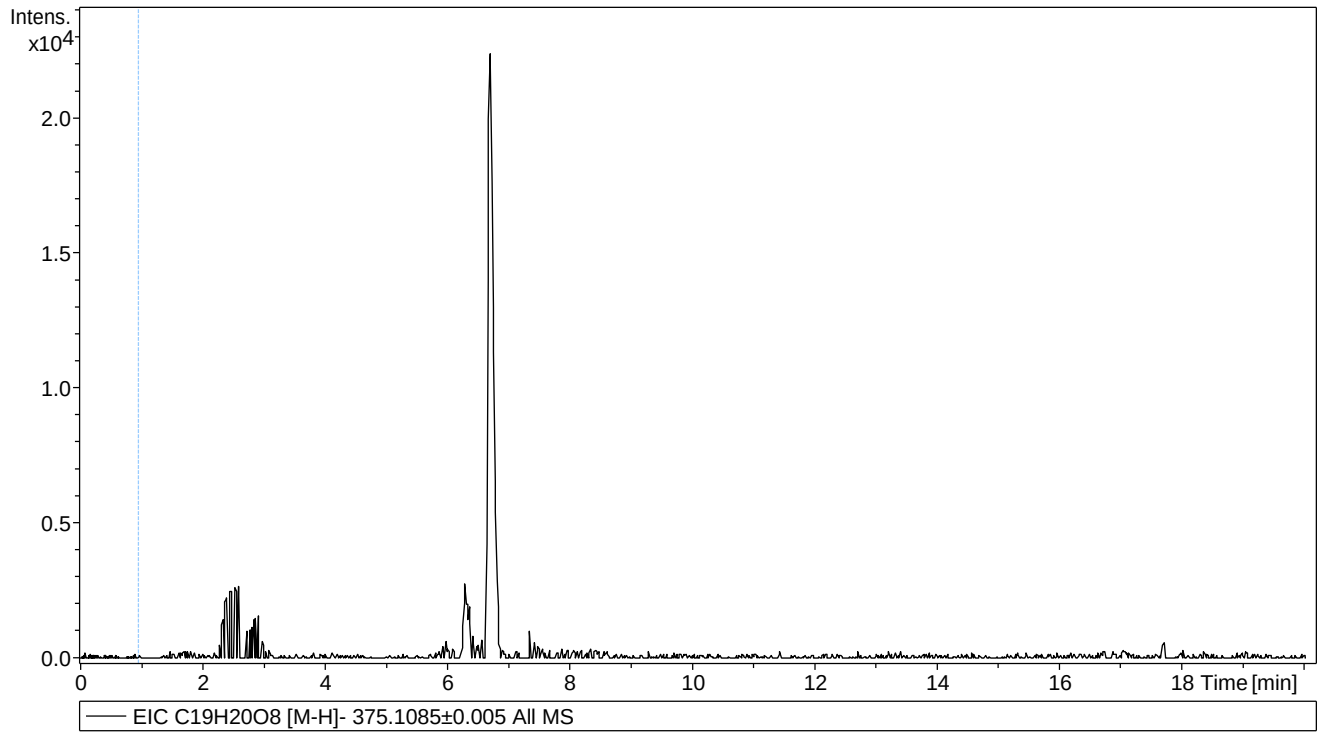
Operator BDAL@DE

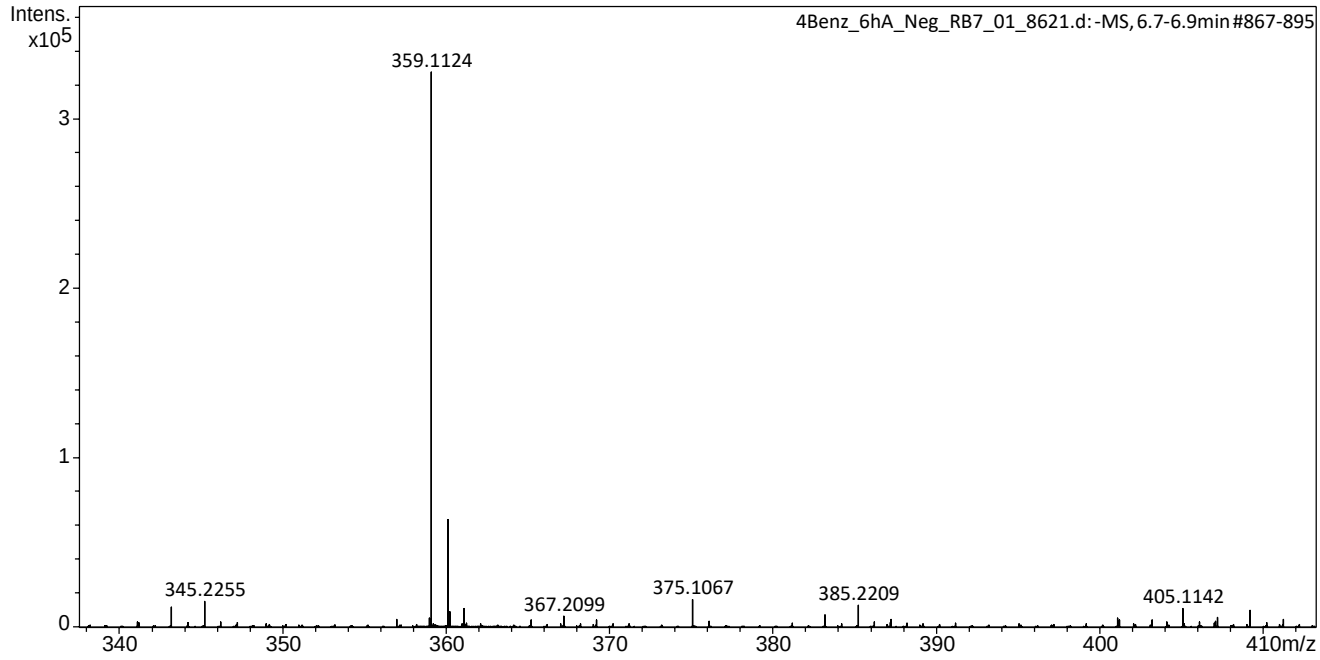
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

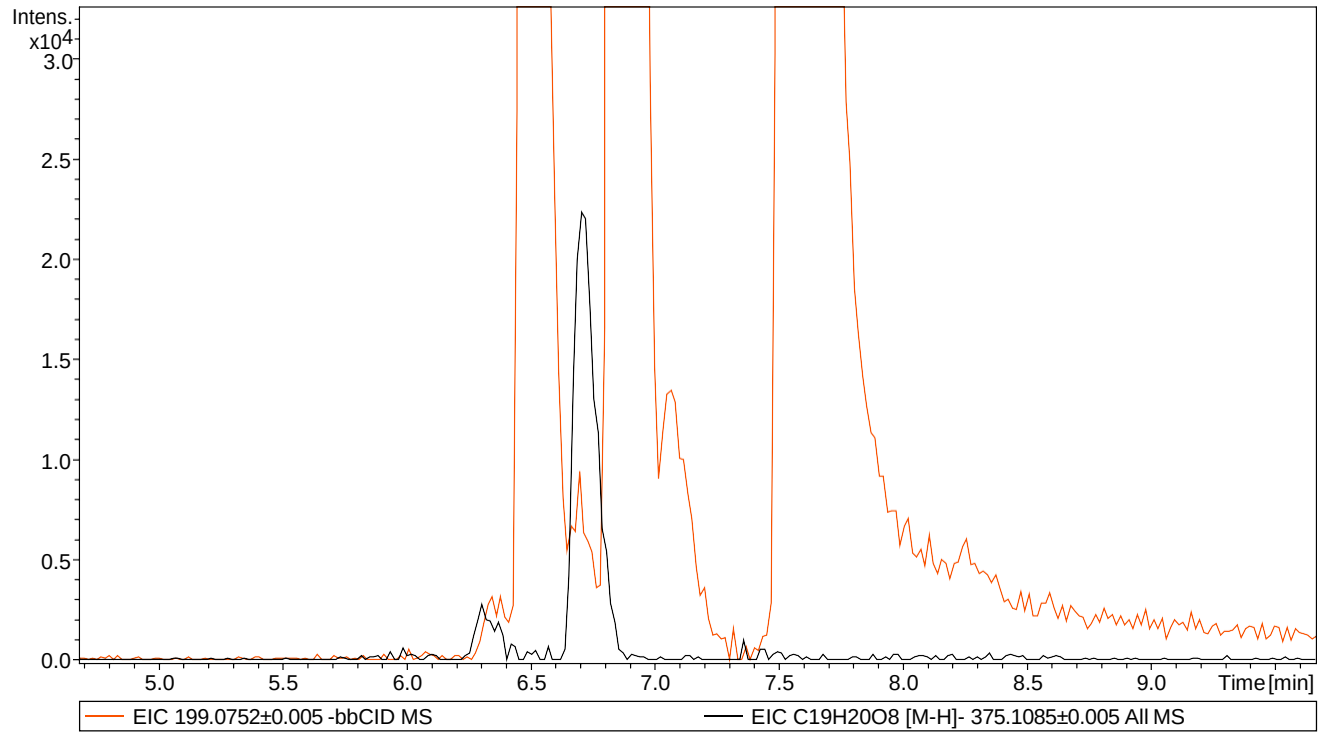
Operator BDAL@DE

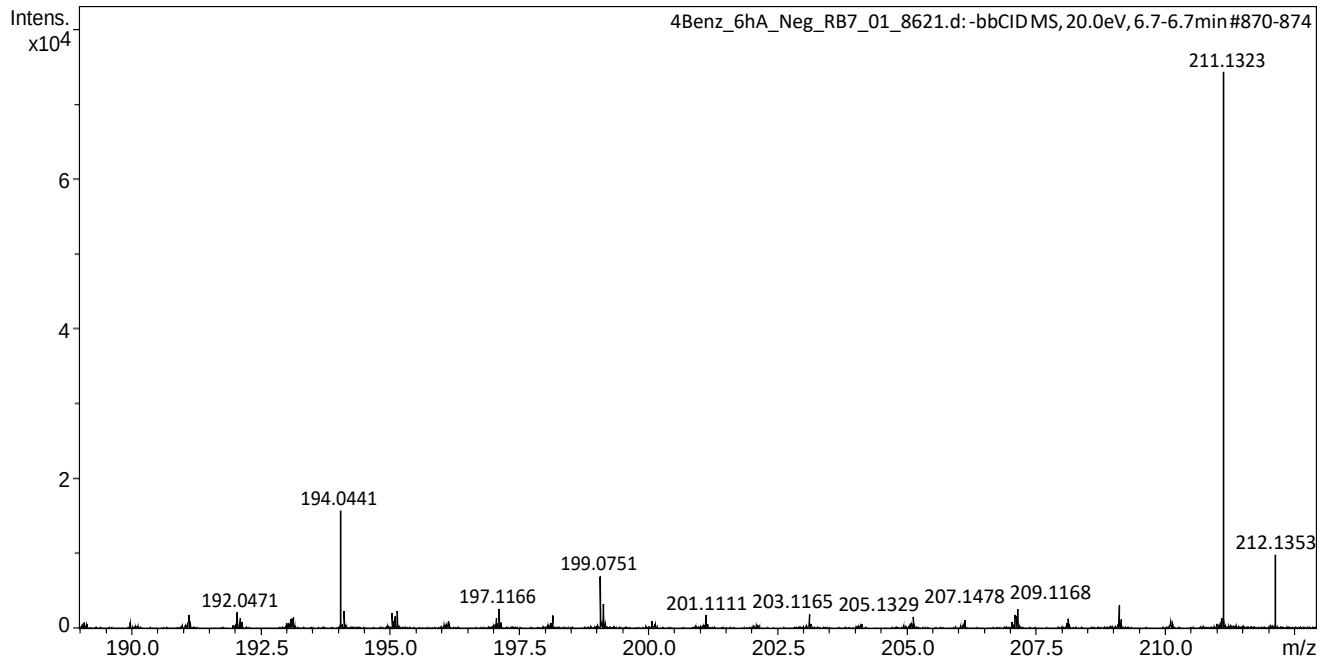
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

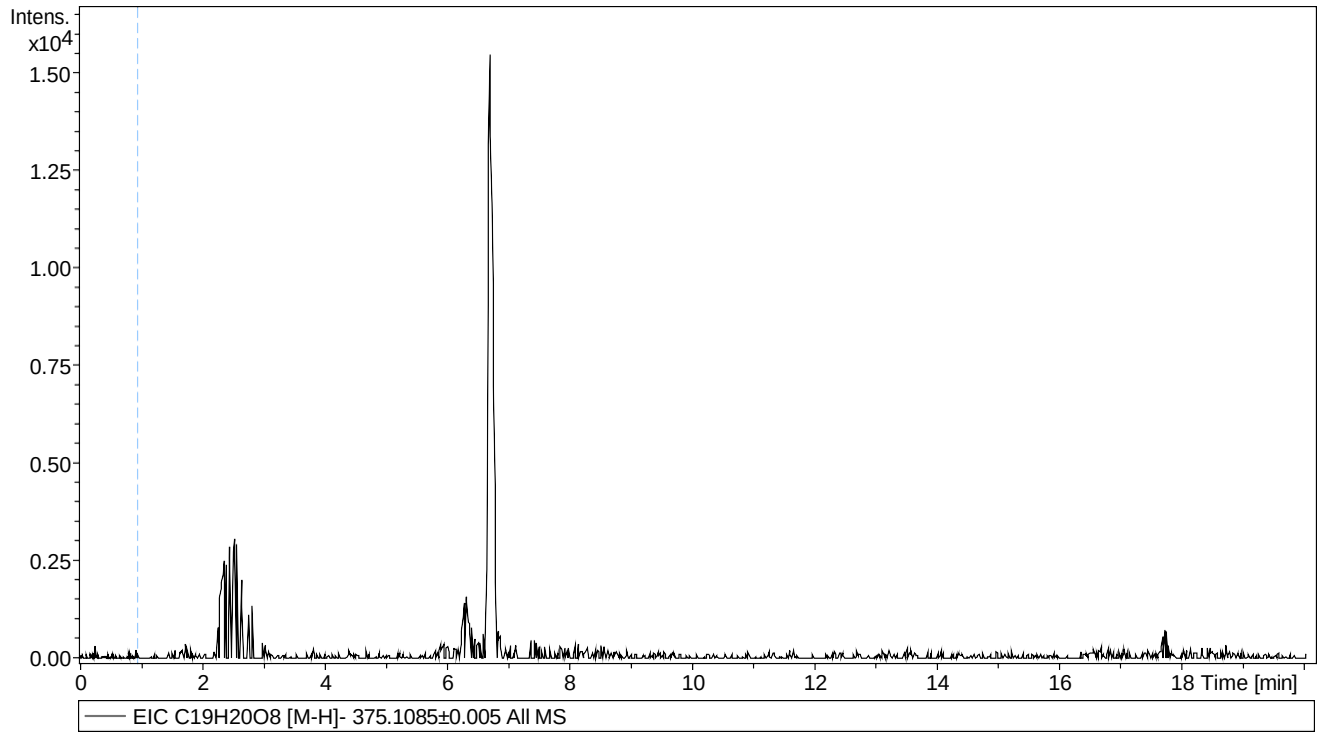
Operator BDAL@DE

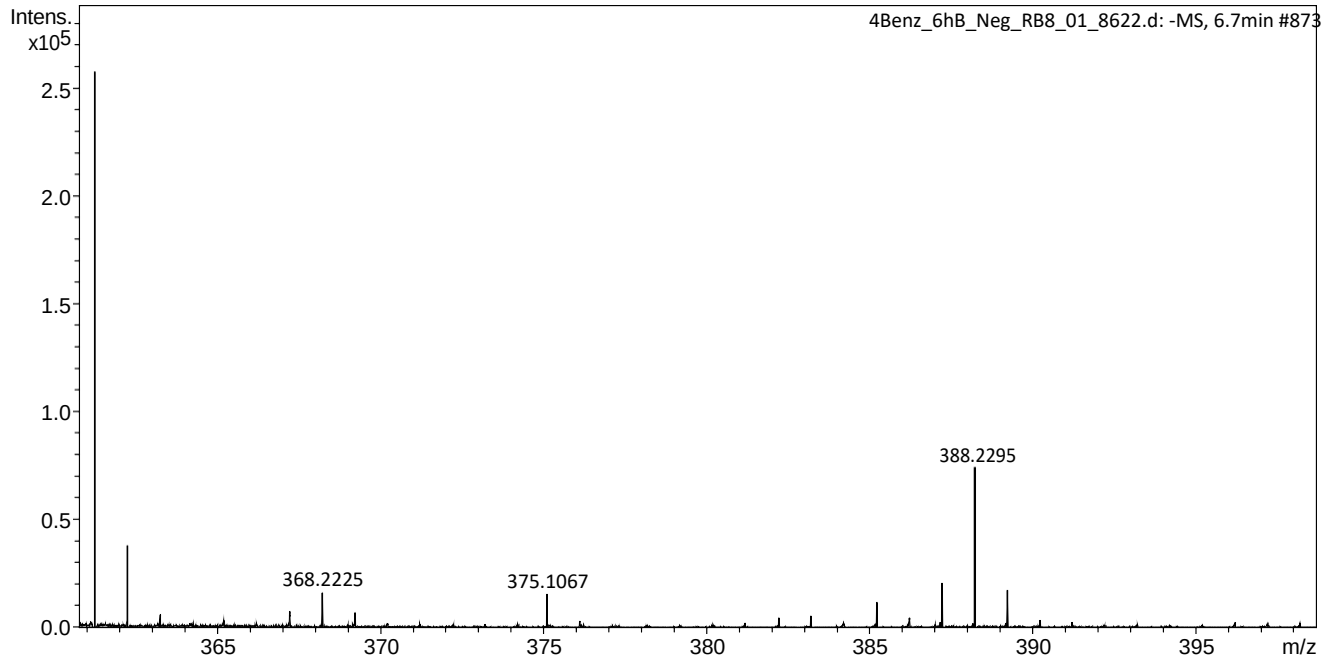
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

Operator BDAL@DE

Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
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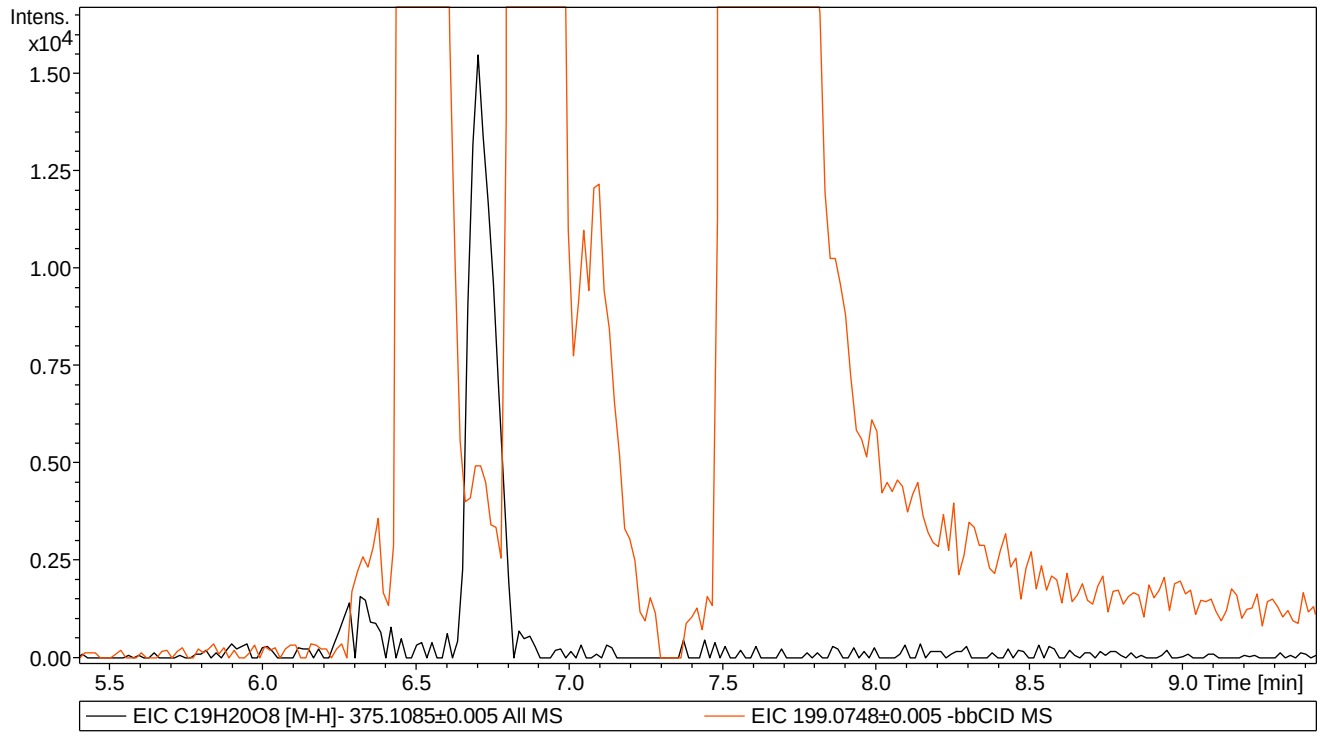
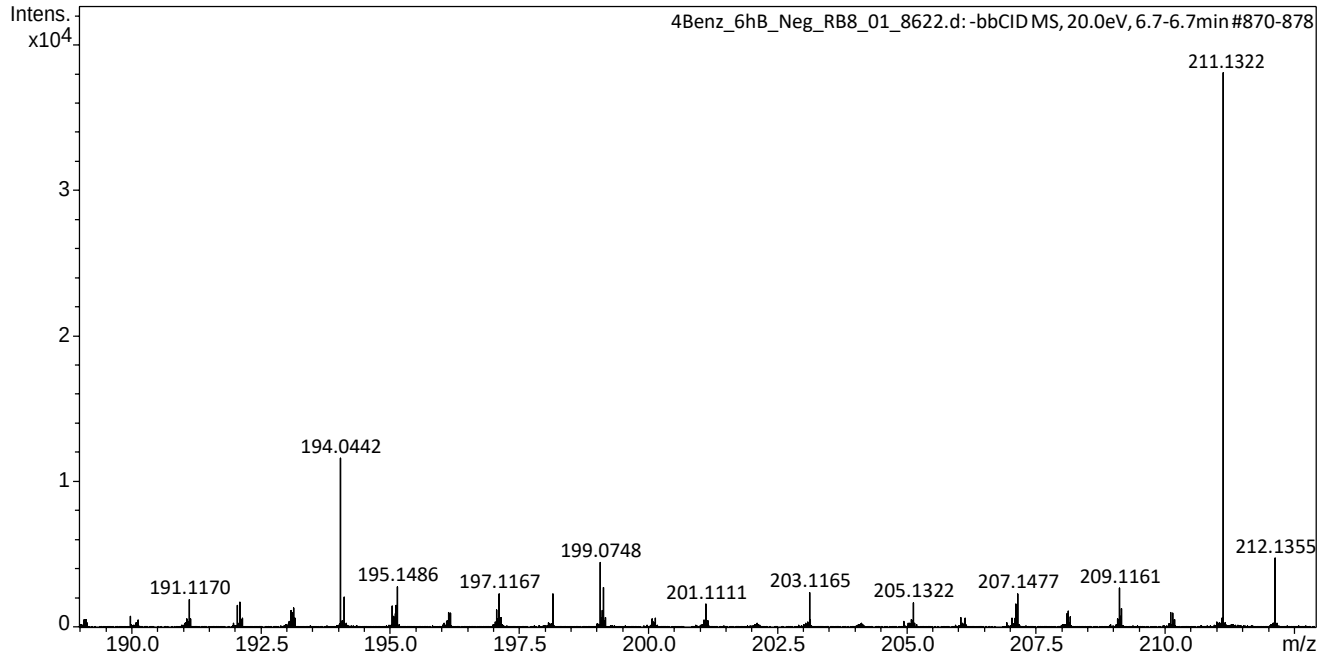




Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

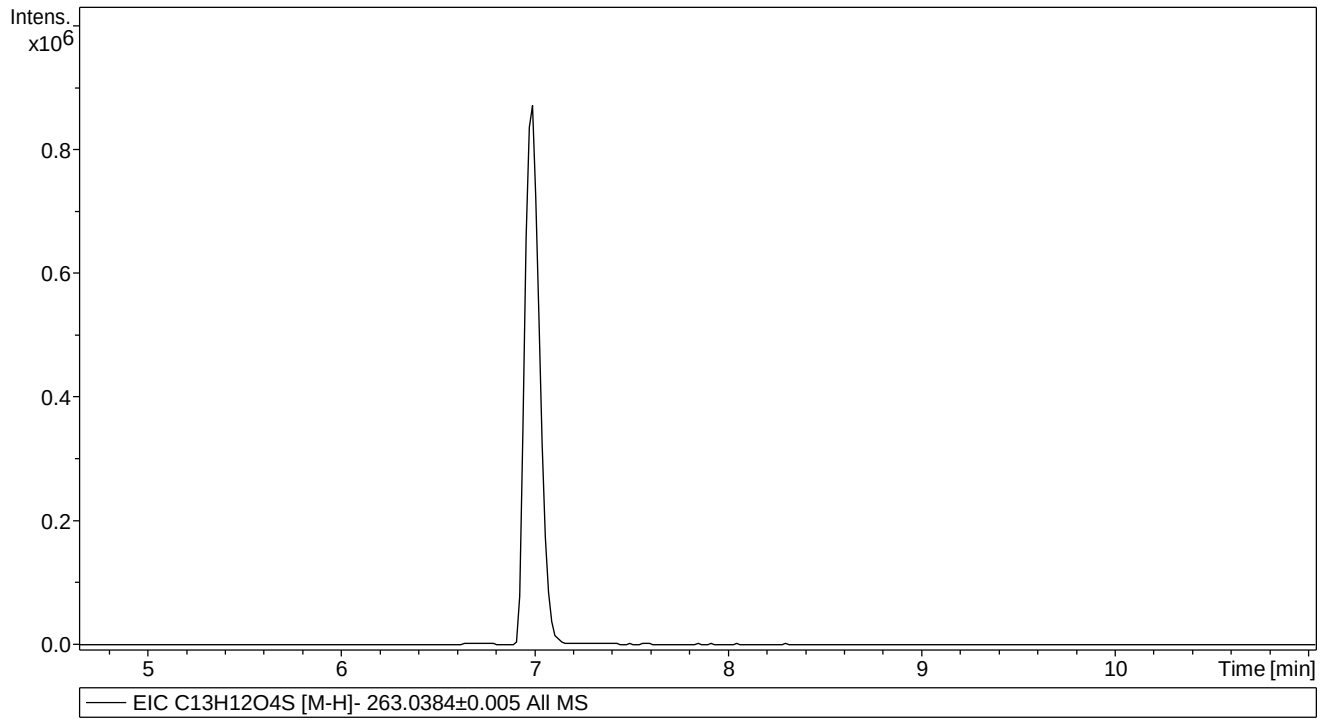
Operator BDAL@DE

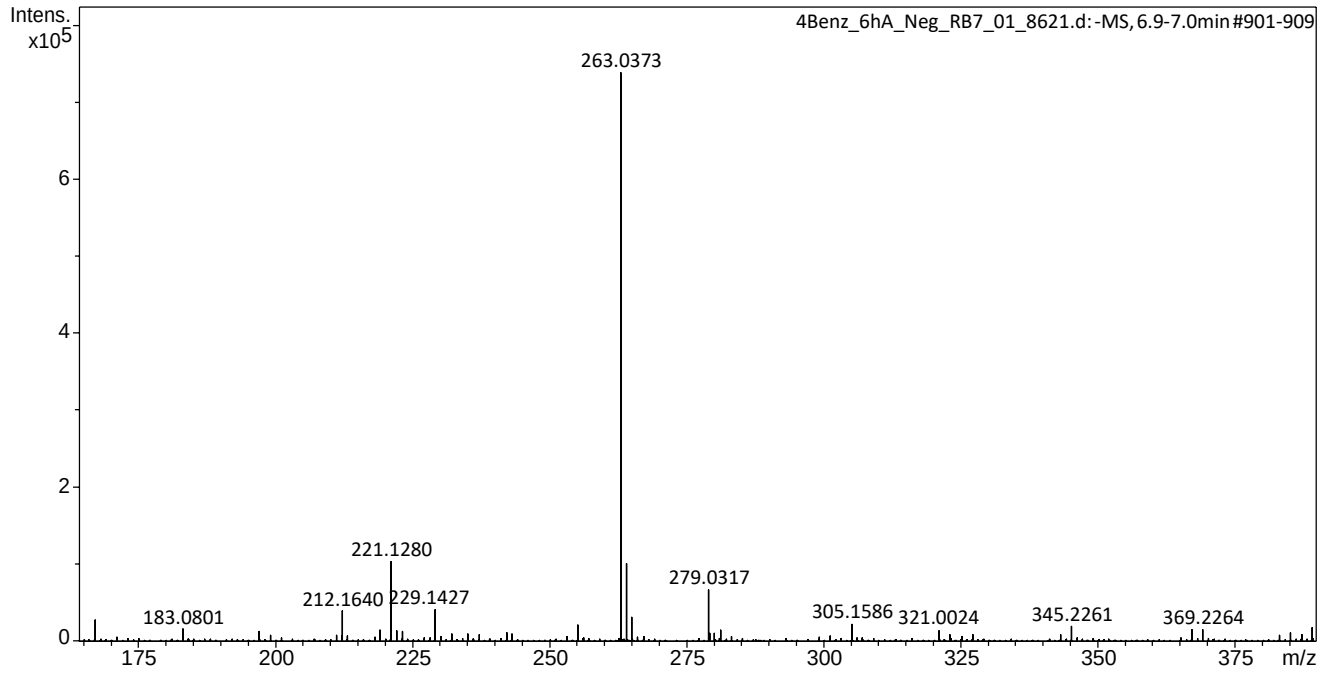
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

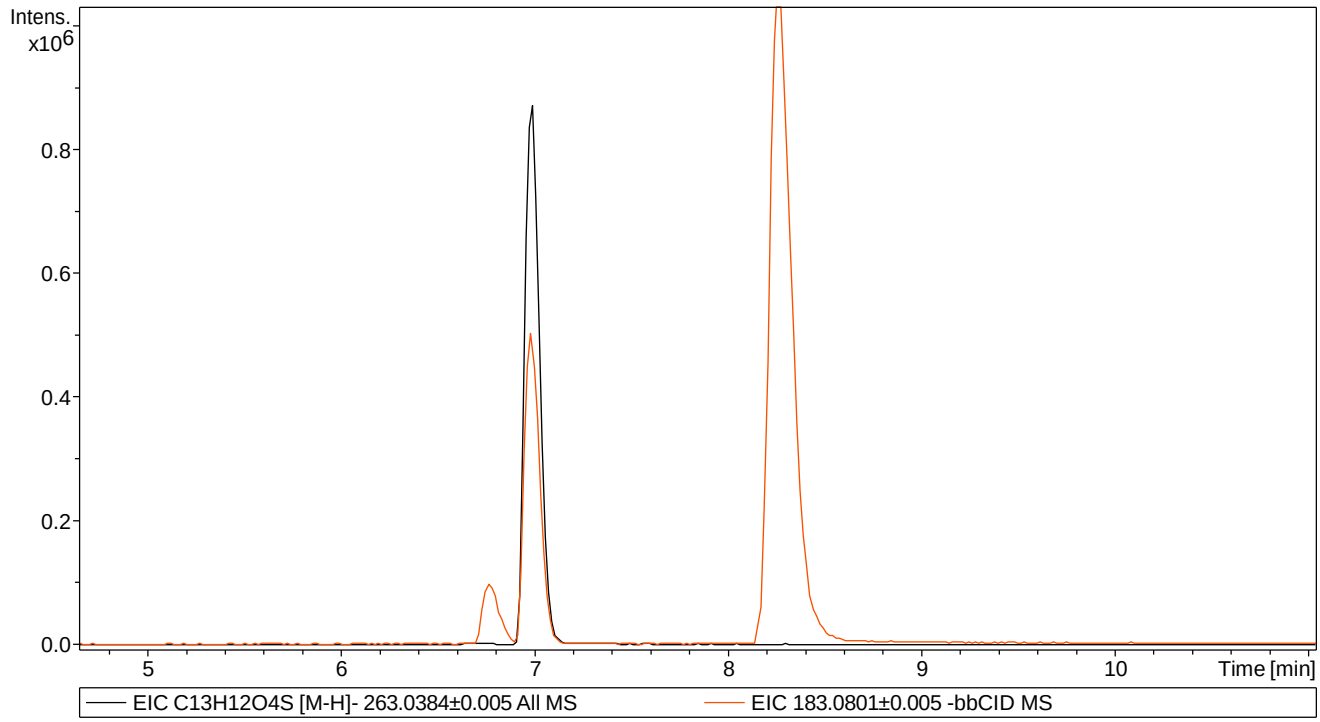
Operator BDAL@DE

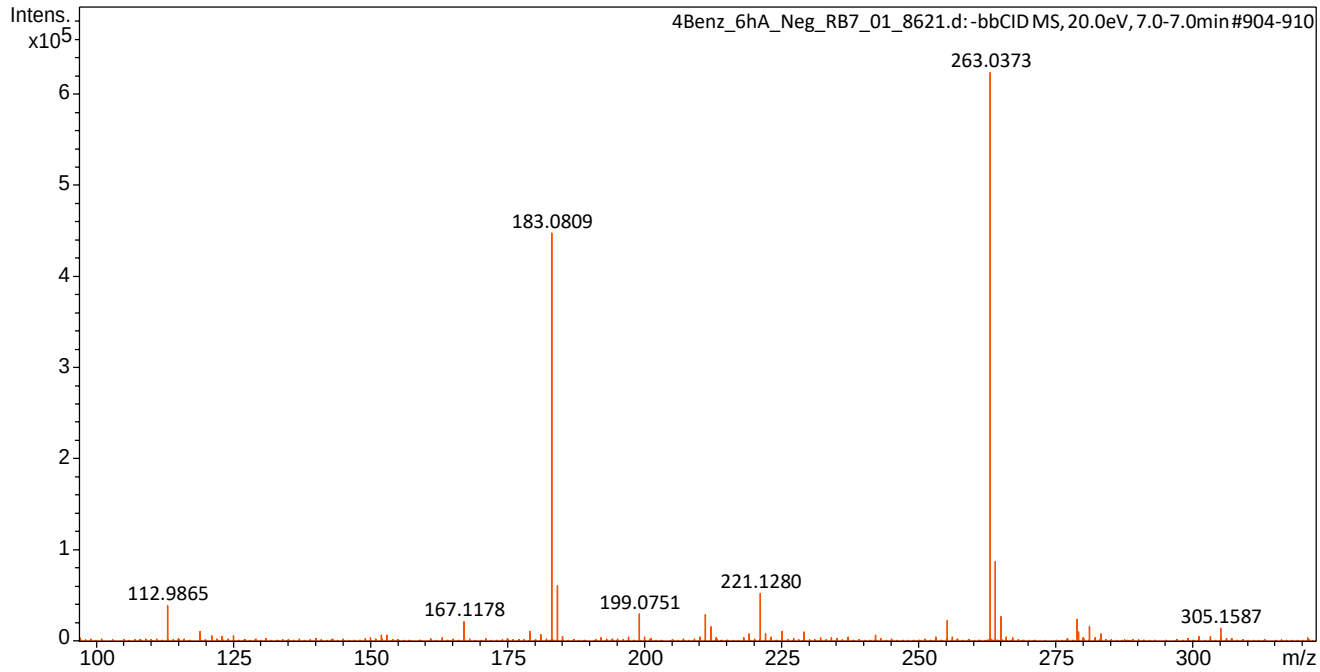
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

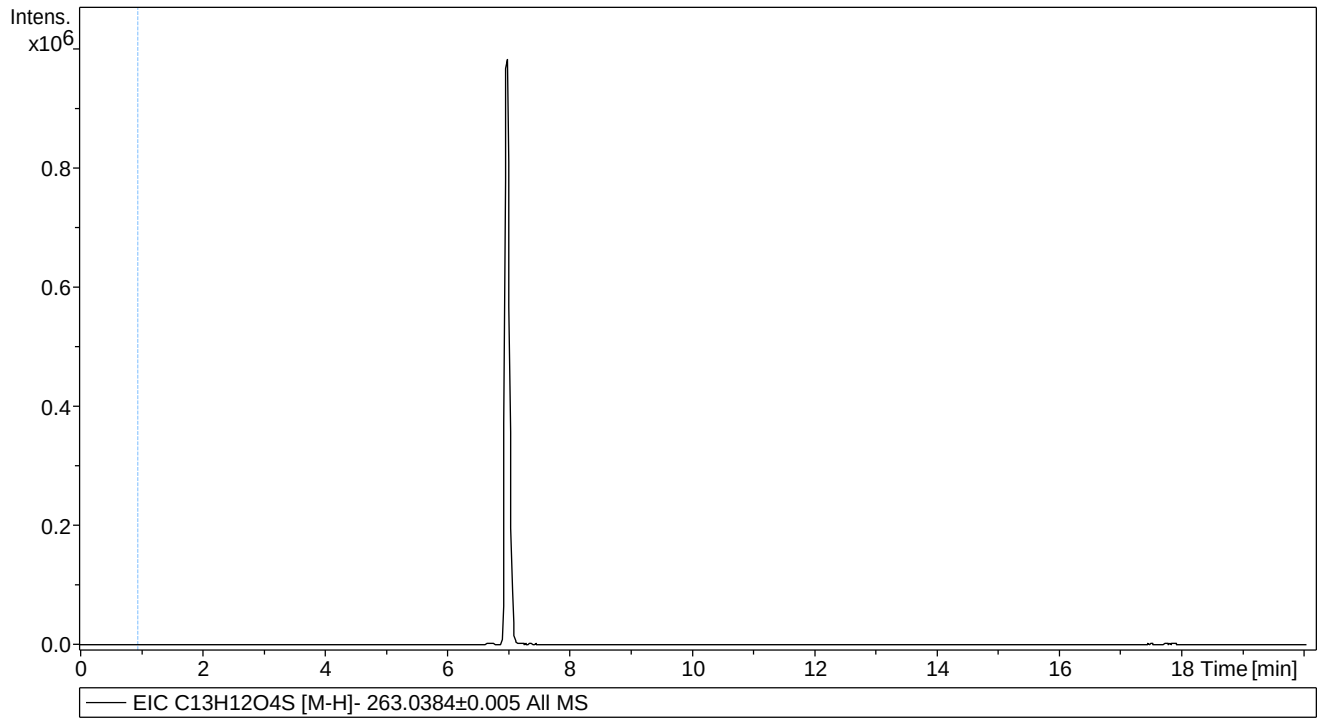
Operator BDAL@DE

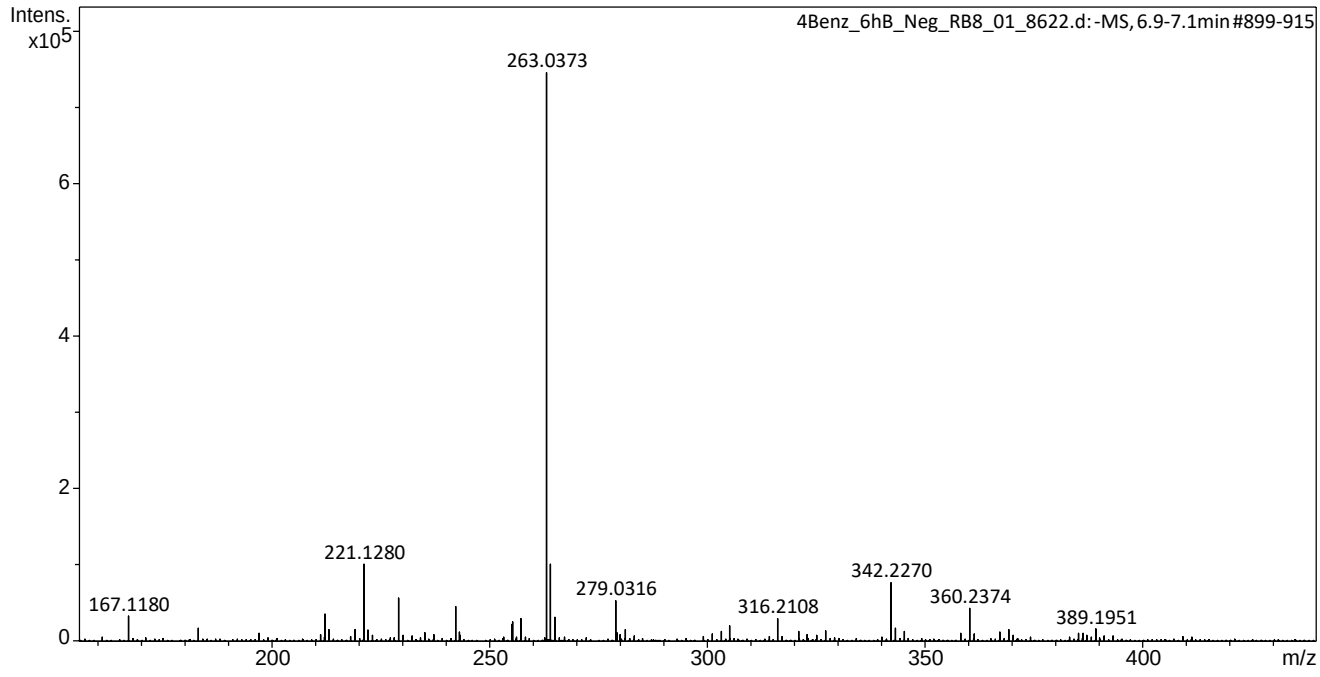
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Analysis Info

Acquisition Date 06/12/2016 21:22:02

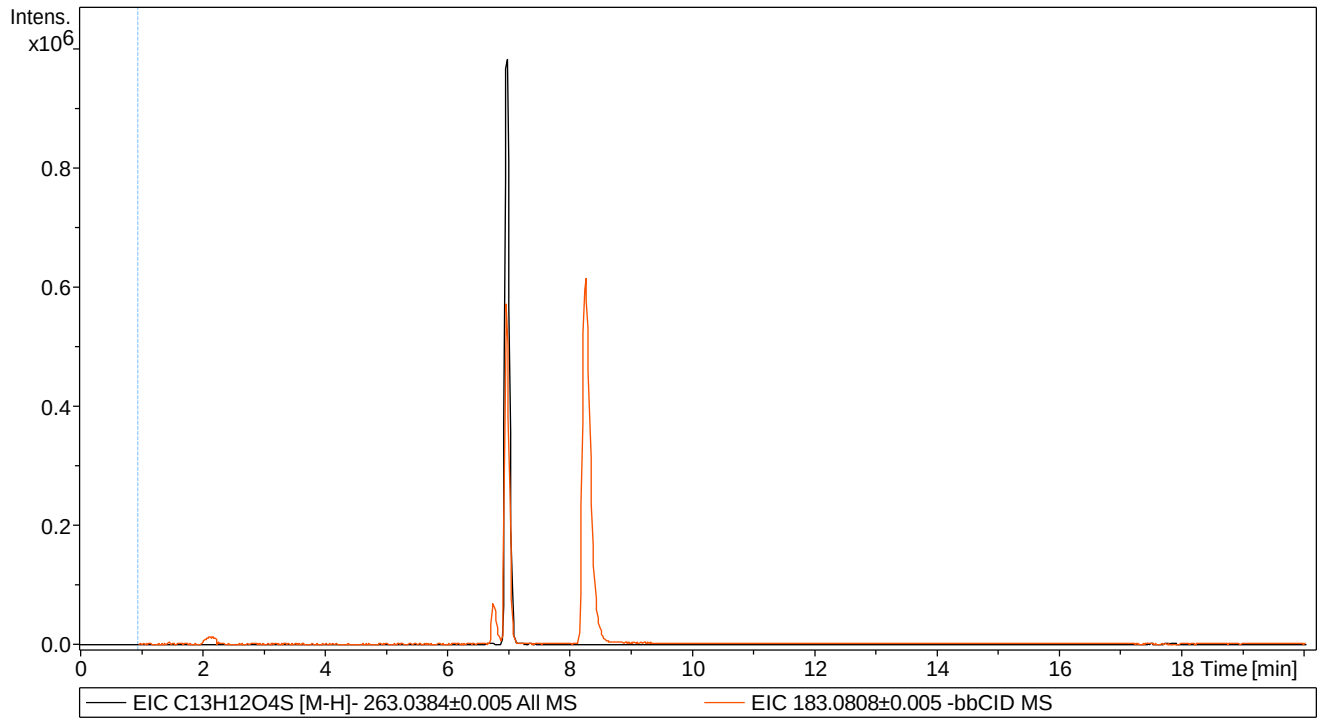
Operator BDAL@DE

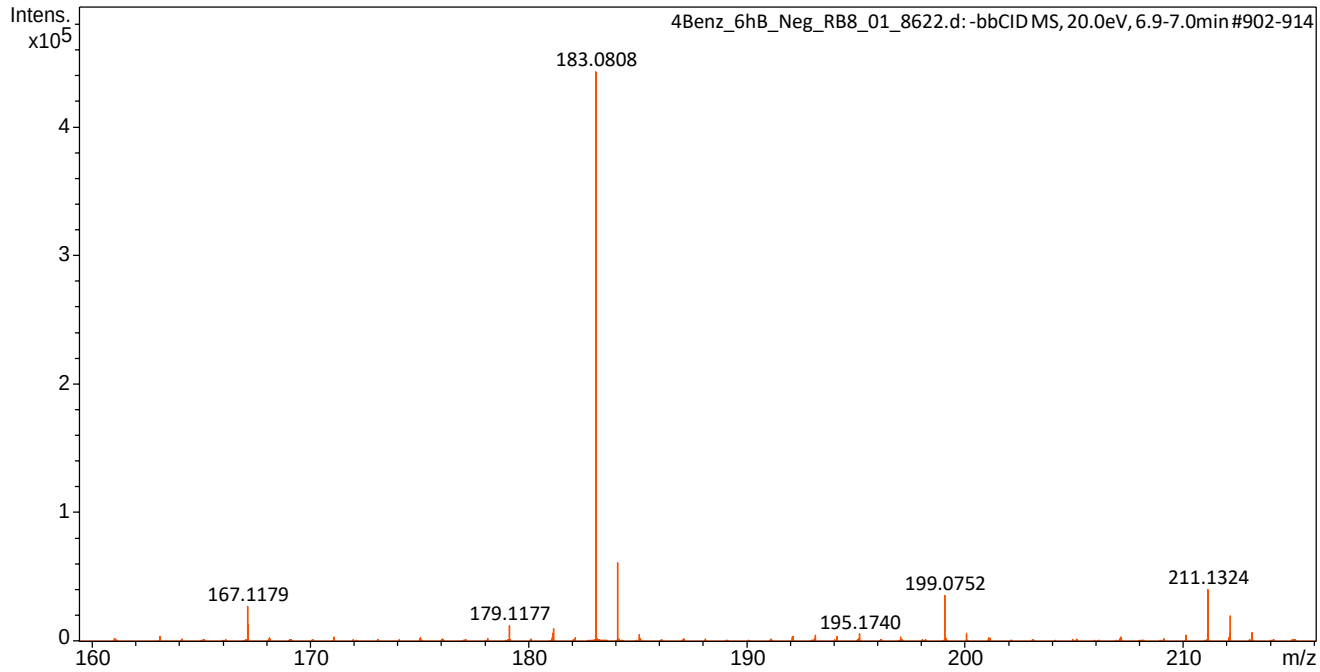
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

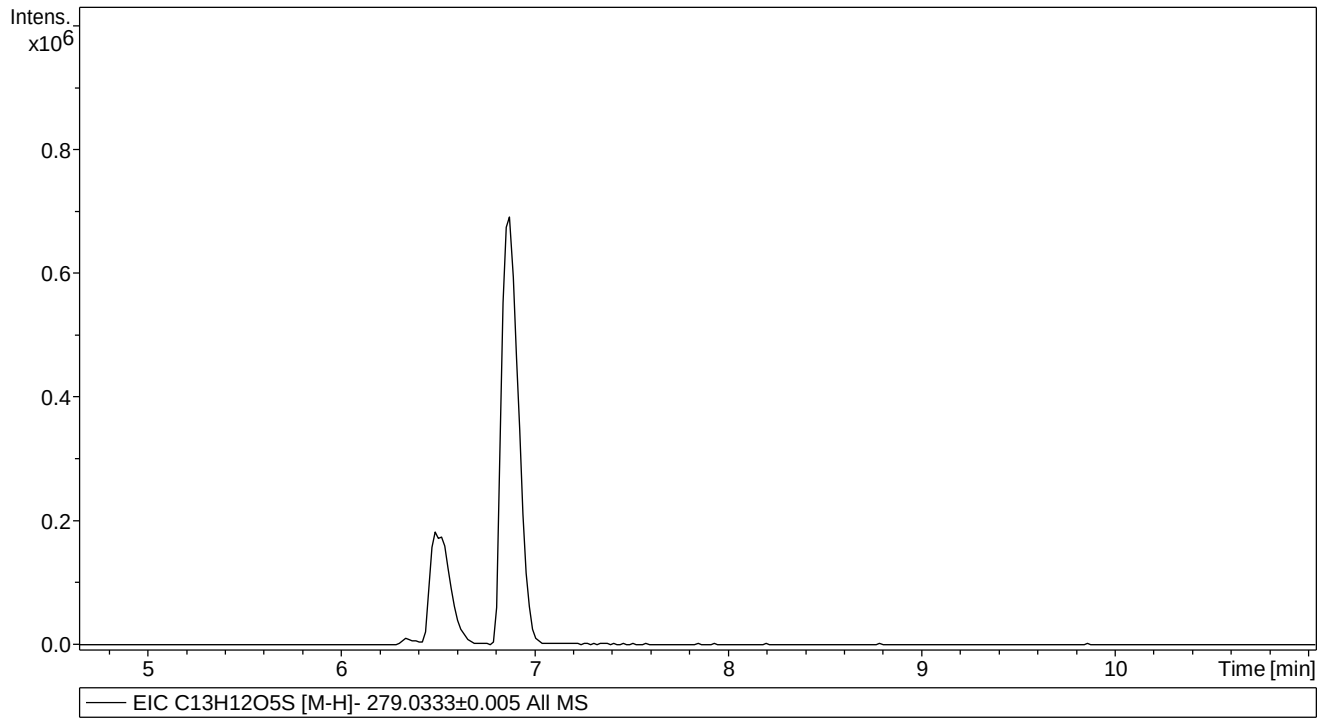
Operator BDAL@DE

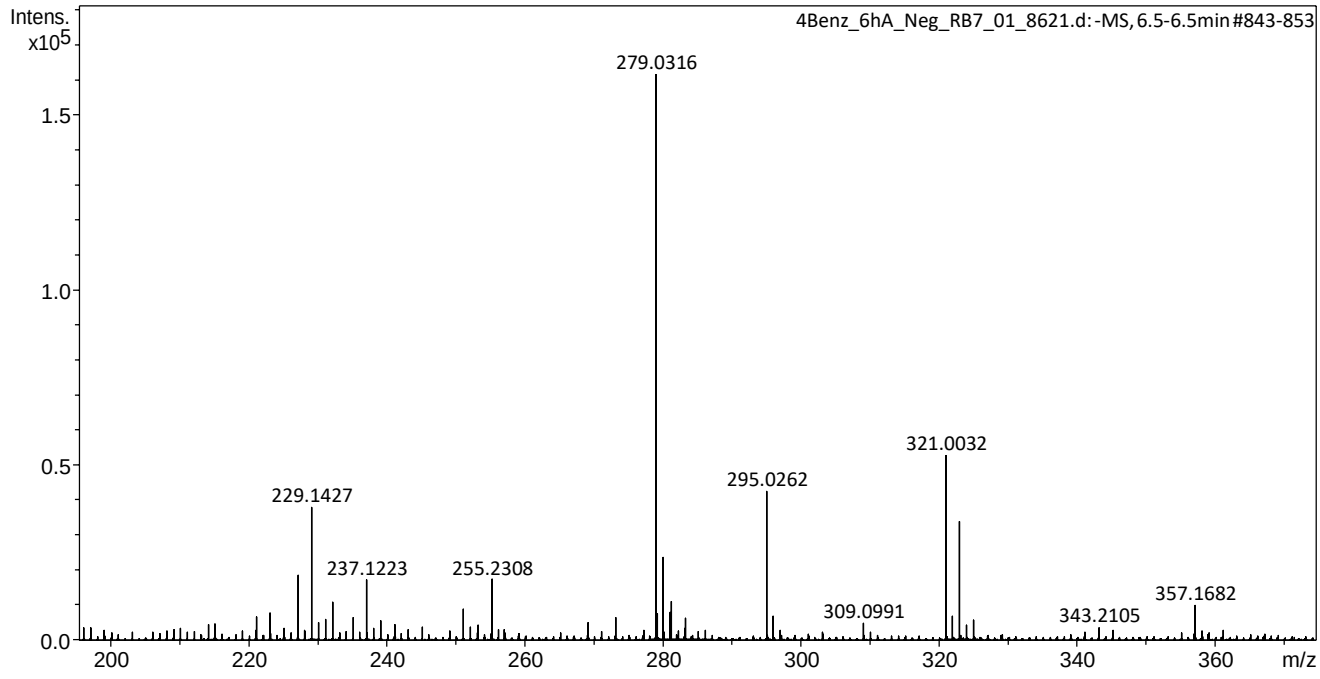
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Analysis Info

Acquisition Date 06/12/2016 21:00:46

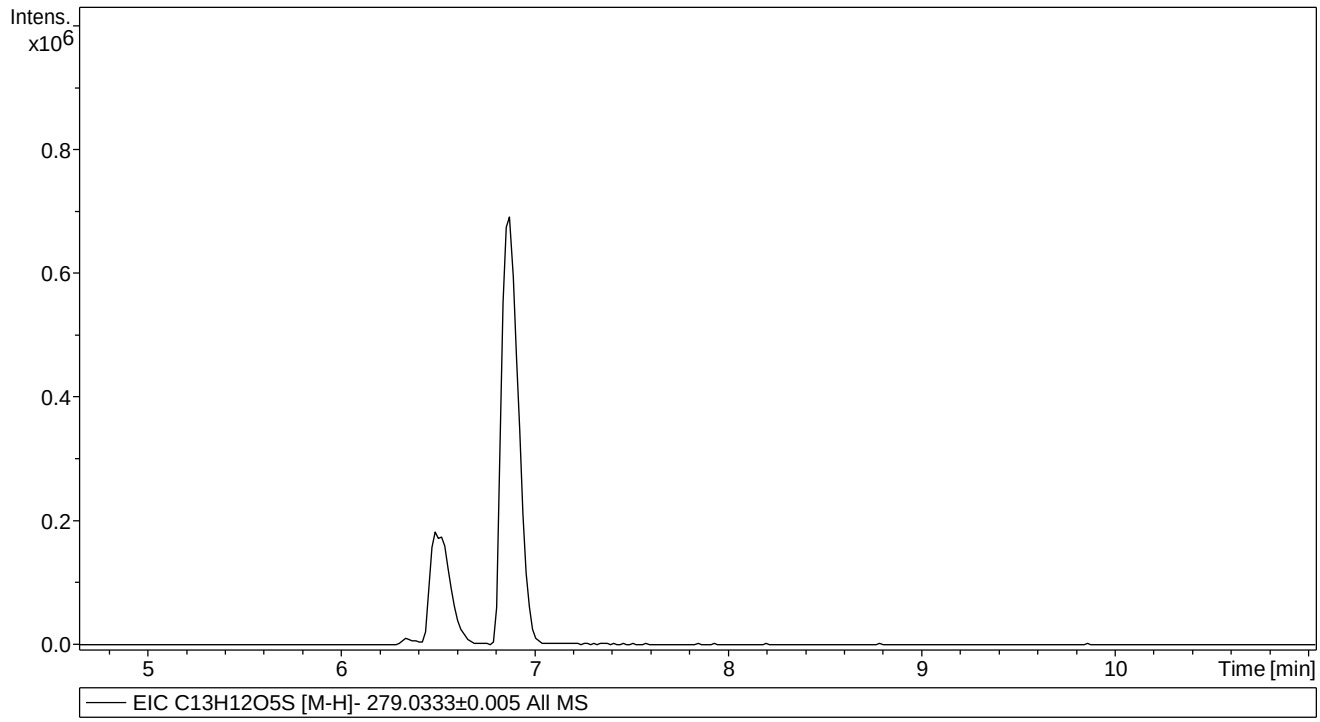
Operator BDAL@DE

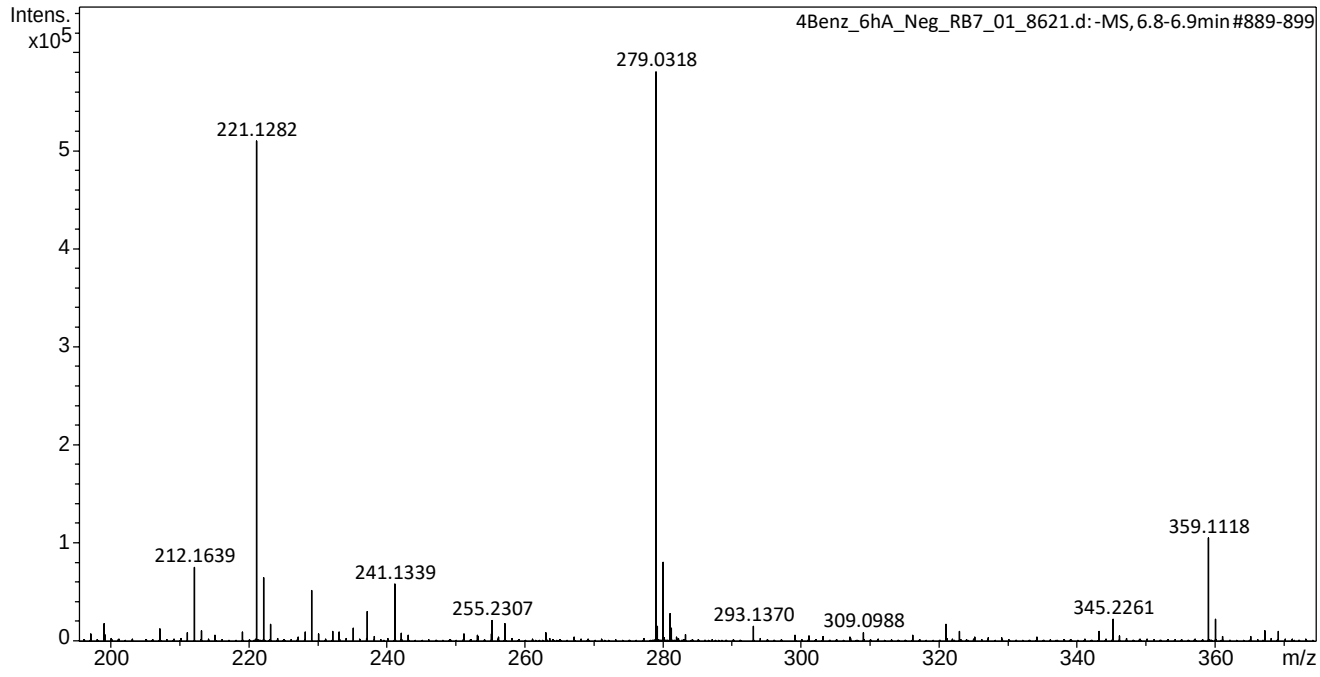
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

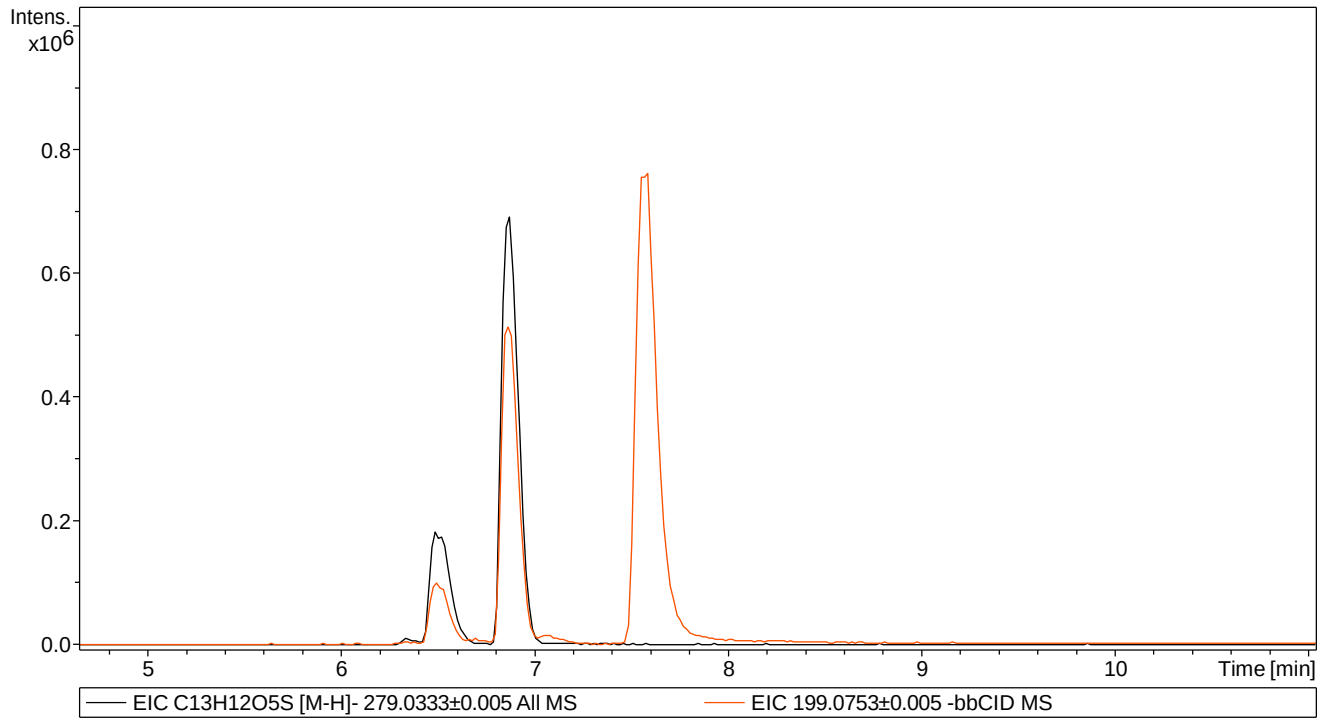
Operator BDAL@DE

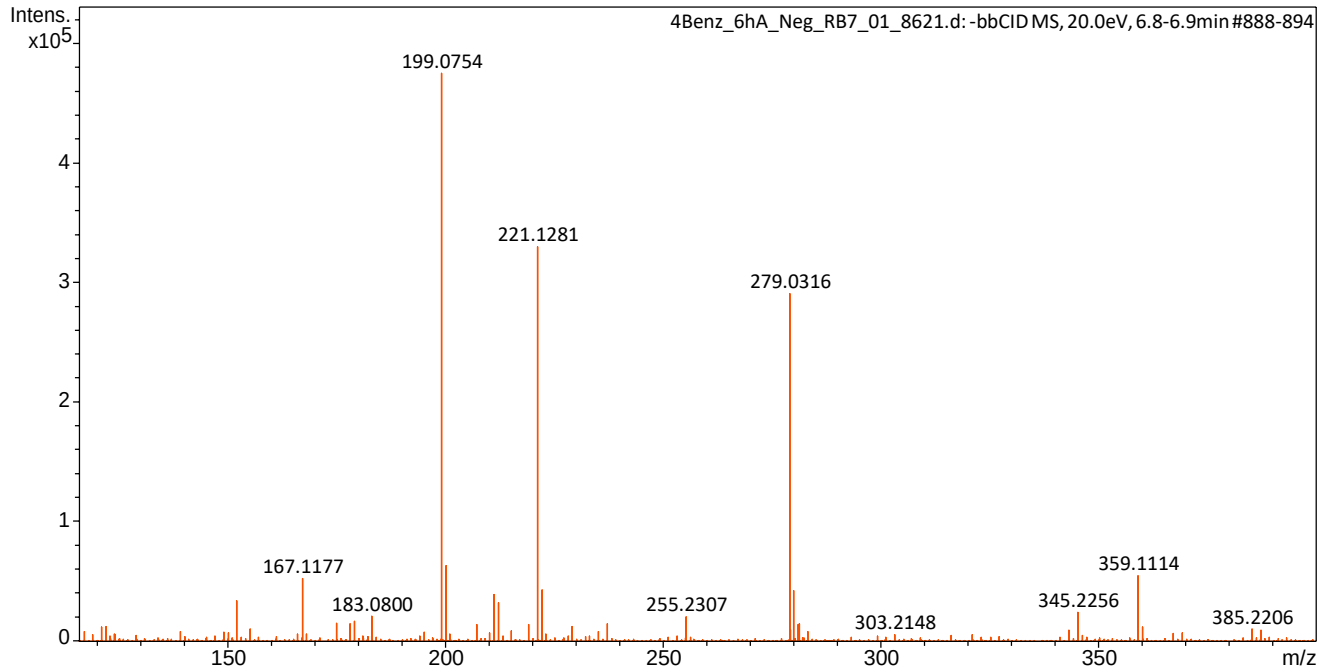
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

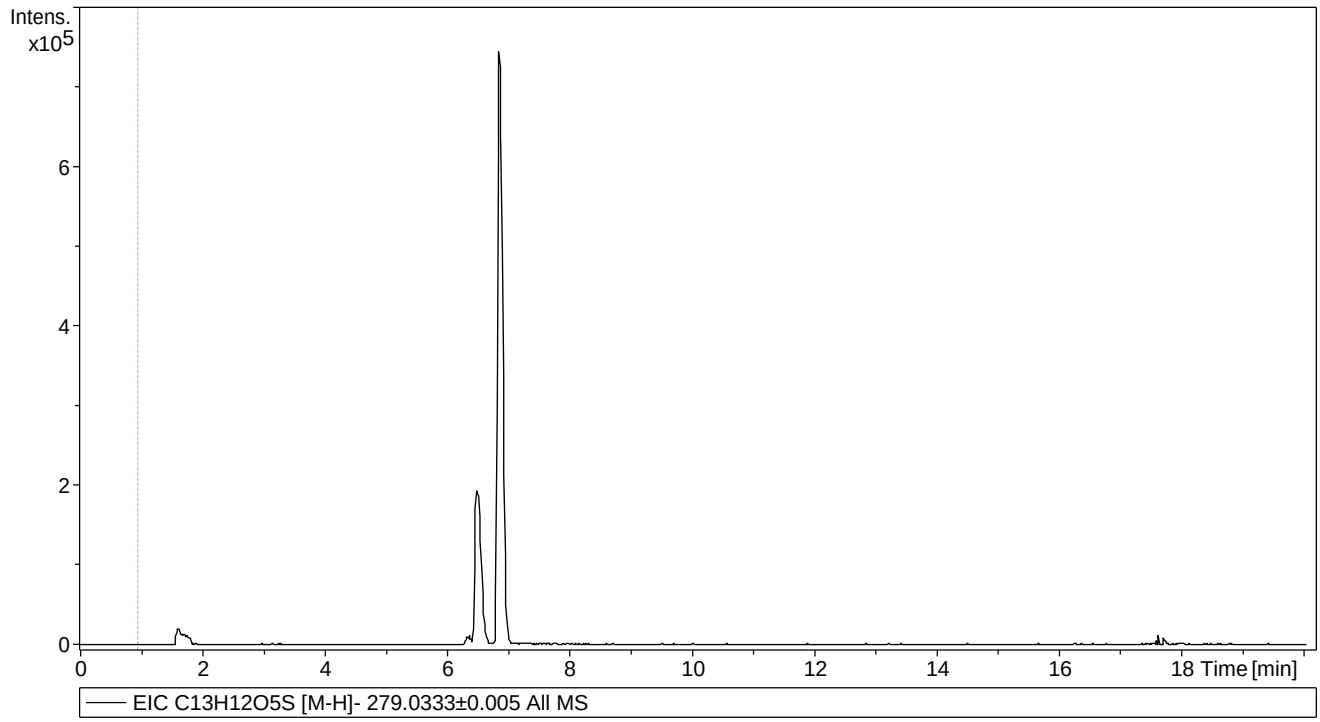
Operator BDAL@DE

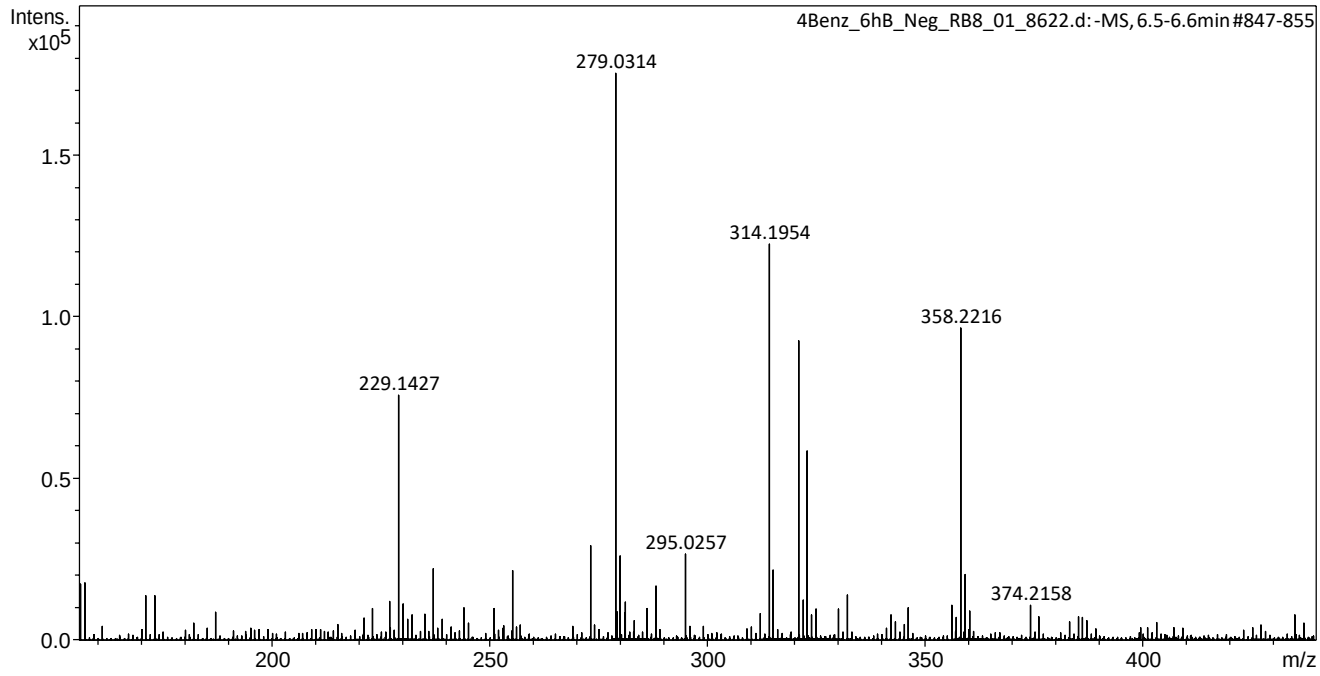
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Analysis Info

Acquisition Date 06/12/2016 21:22:02

Operator BDAL@DE

Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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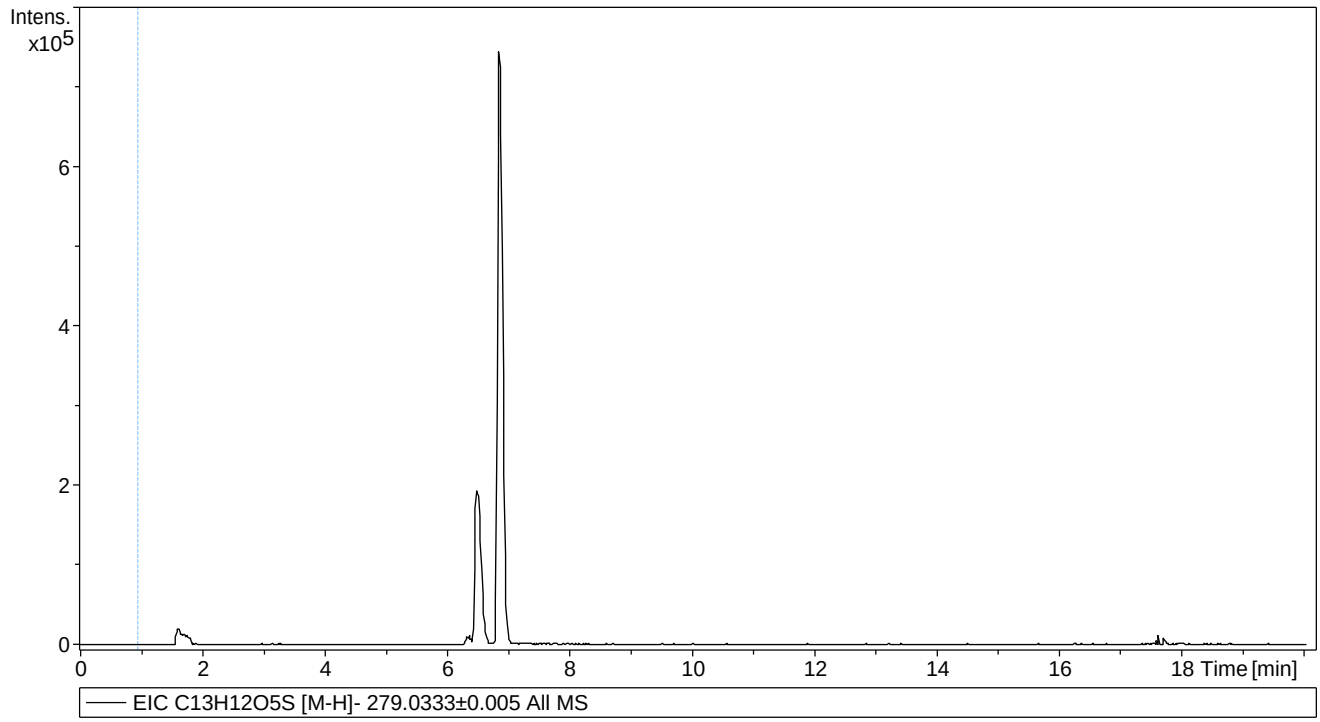
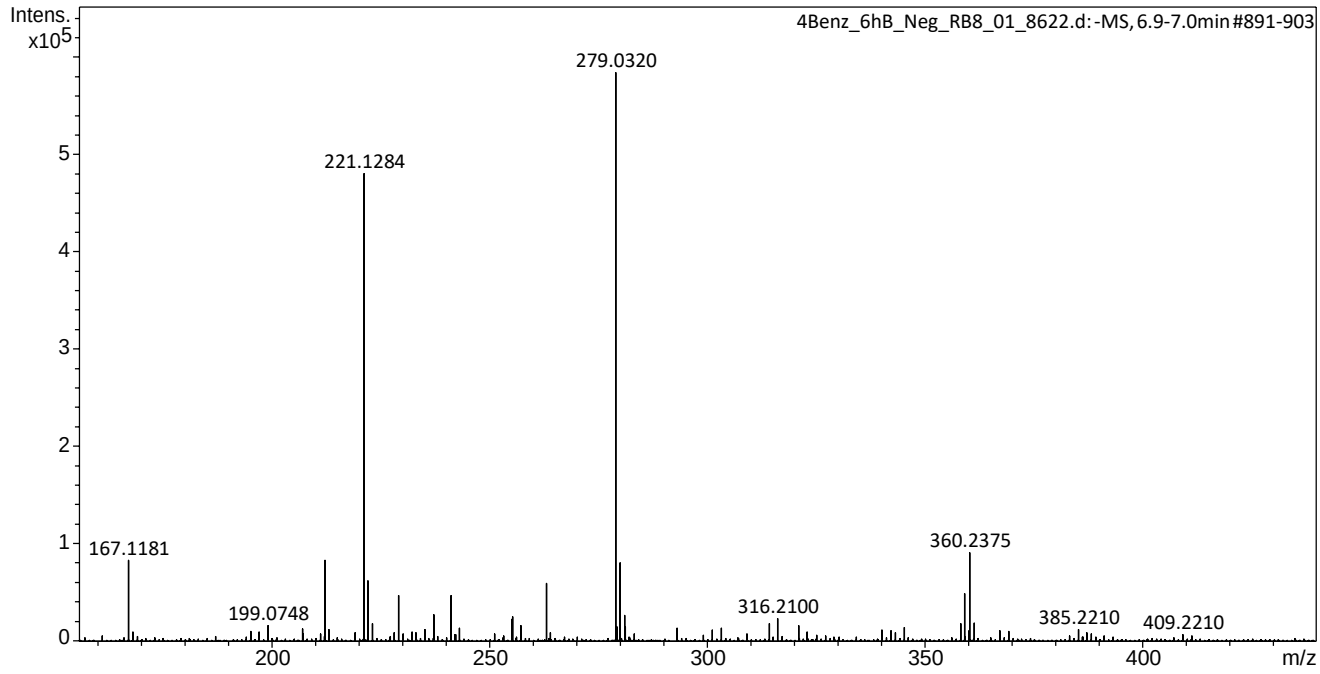




Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

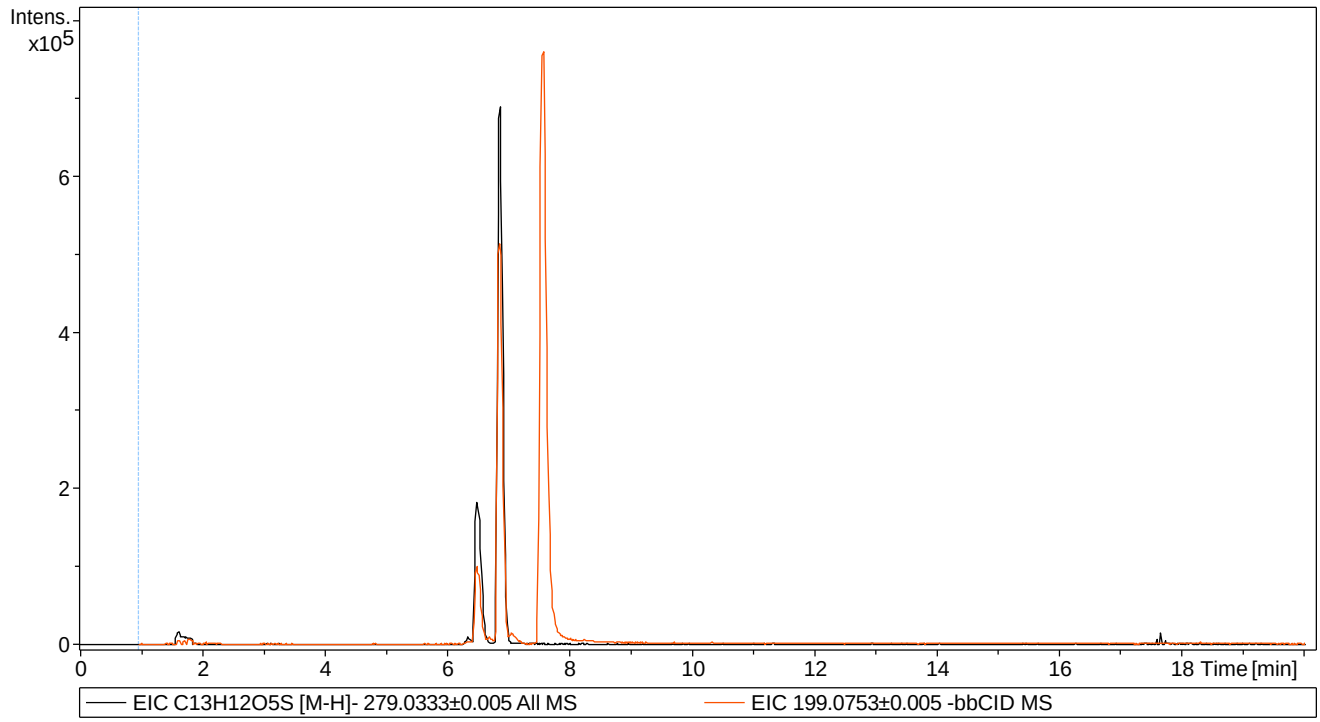
Operator BDAL@DE

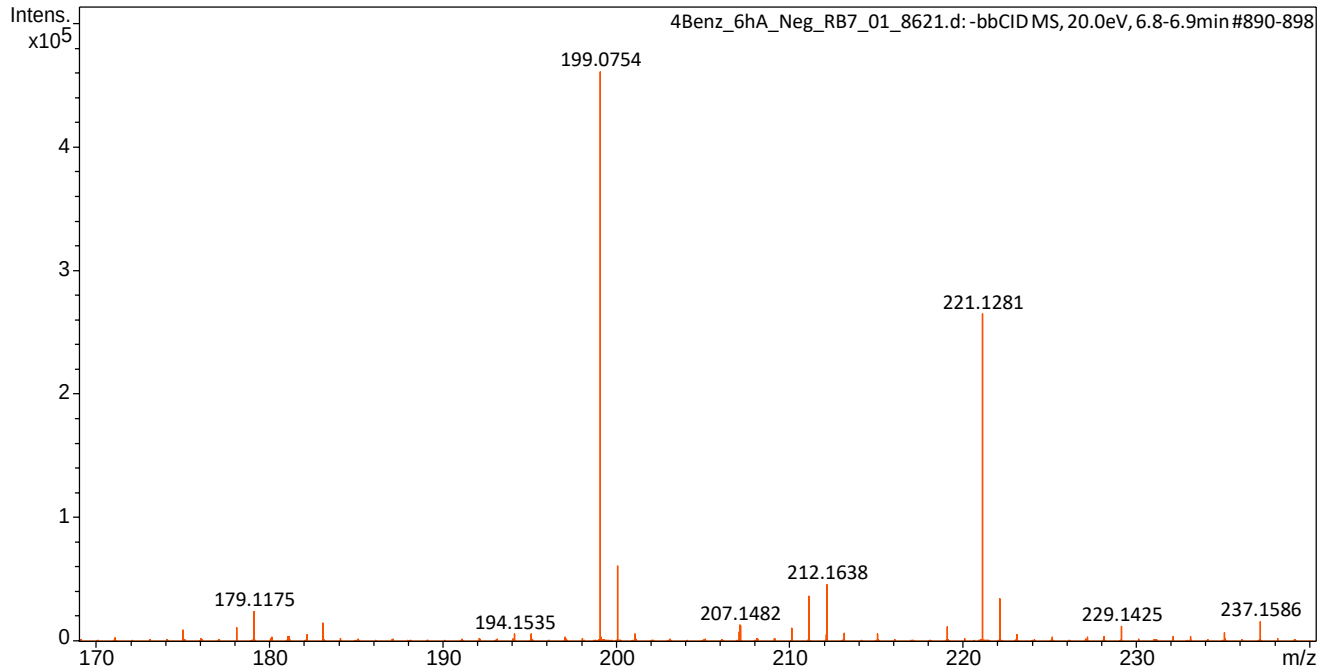
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Analysis Info

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Operator BDAL@DE

Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
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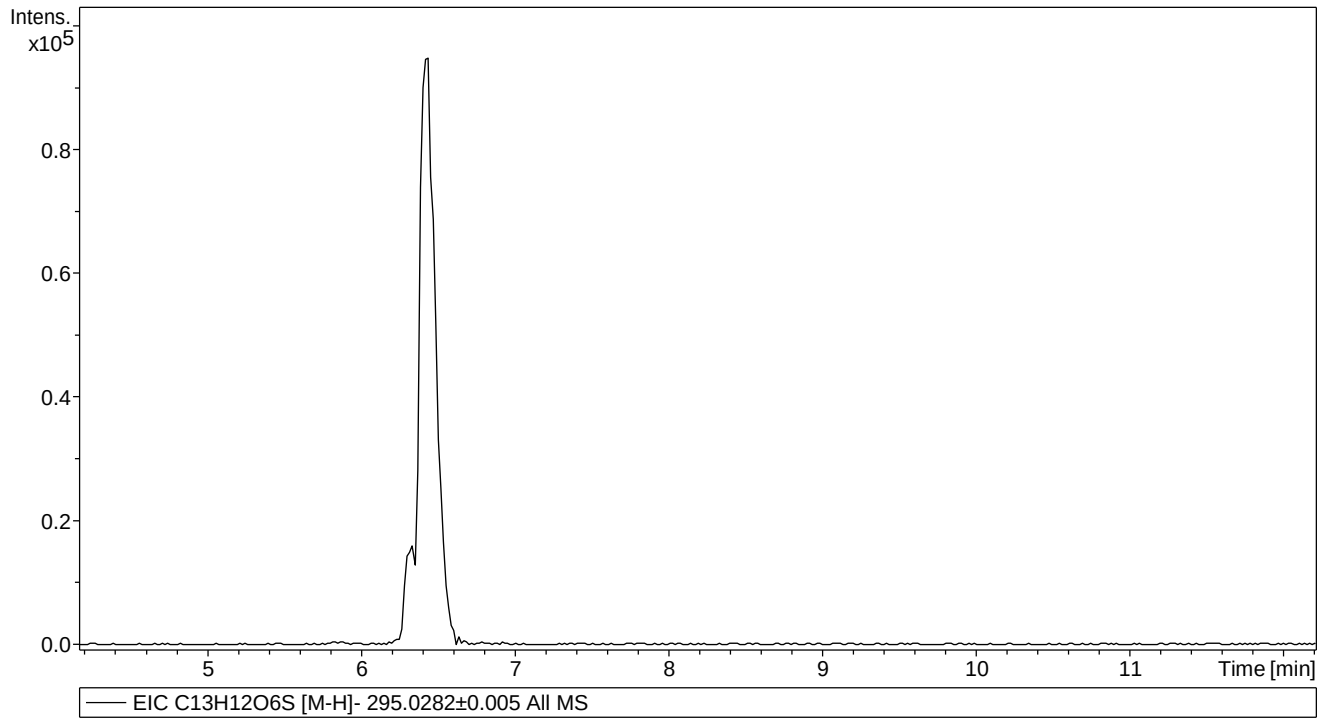
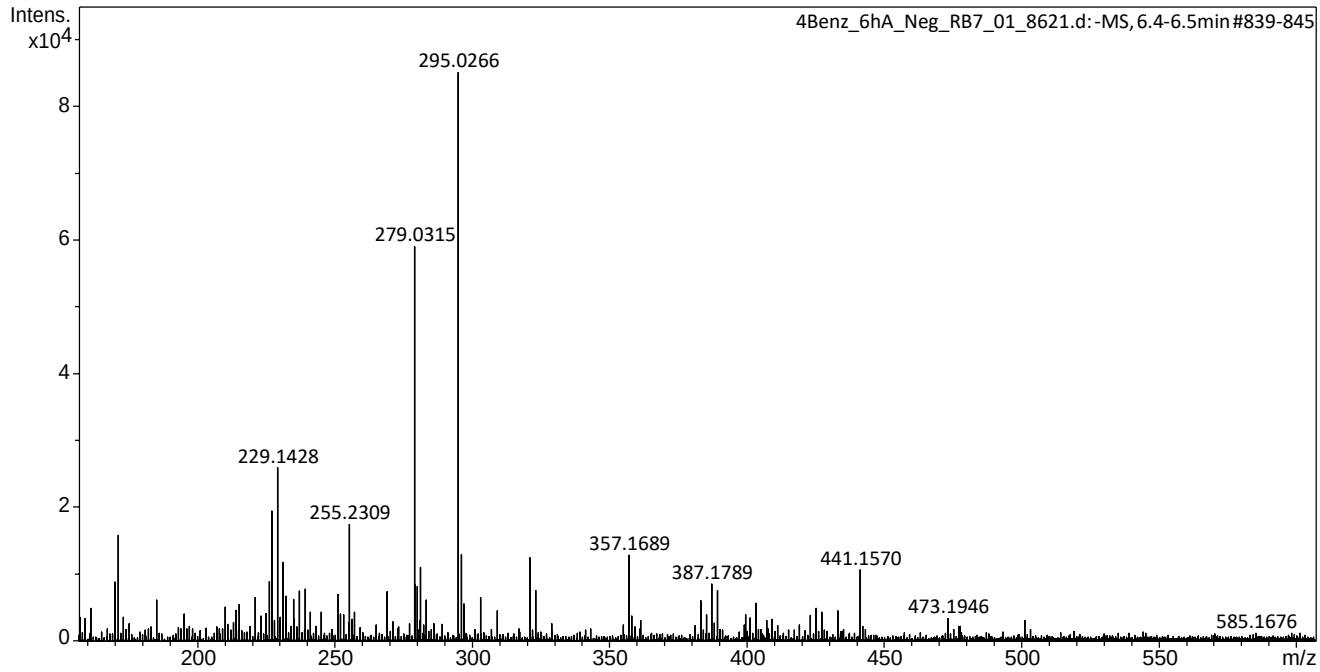




4Benz_6hA_Neg_RB7_01_8621.d

Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

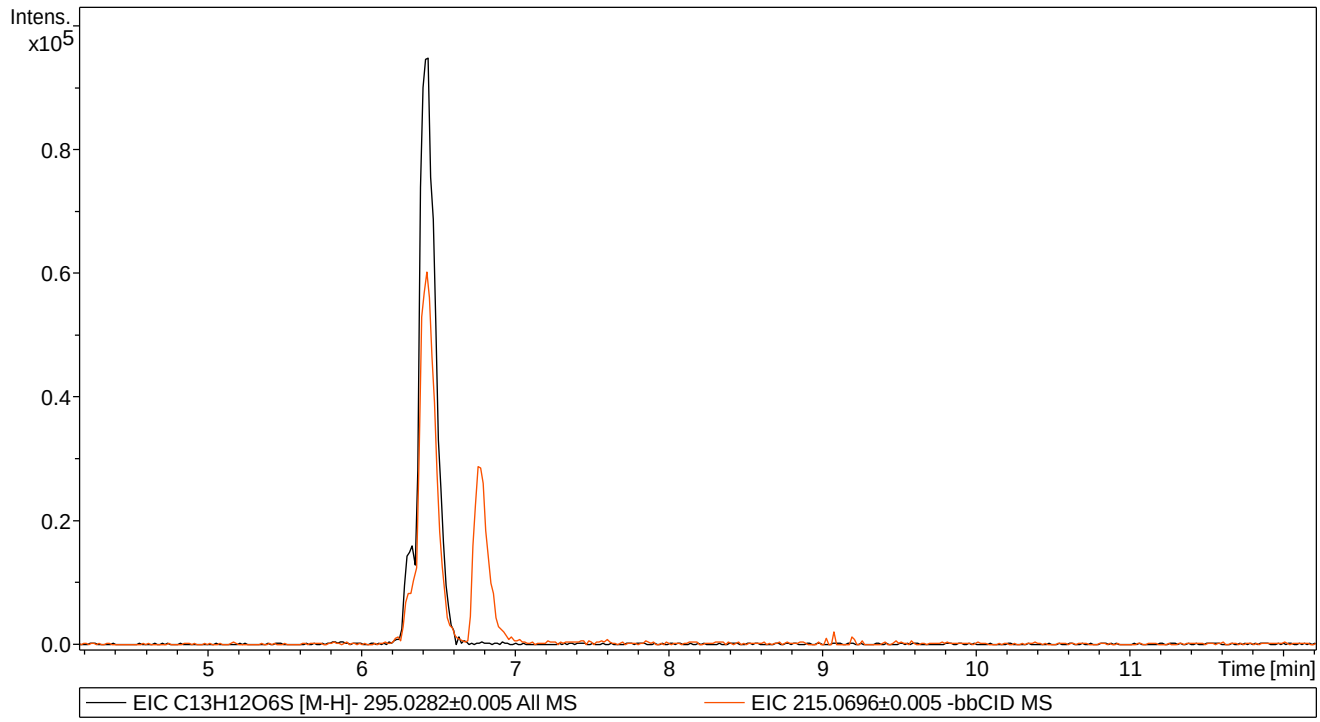
Operator BDAL@DE

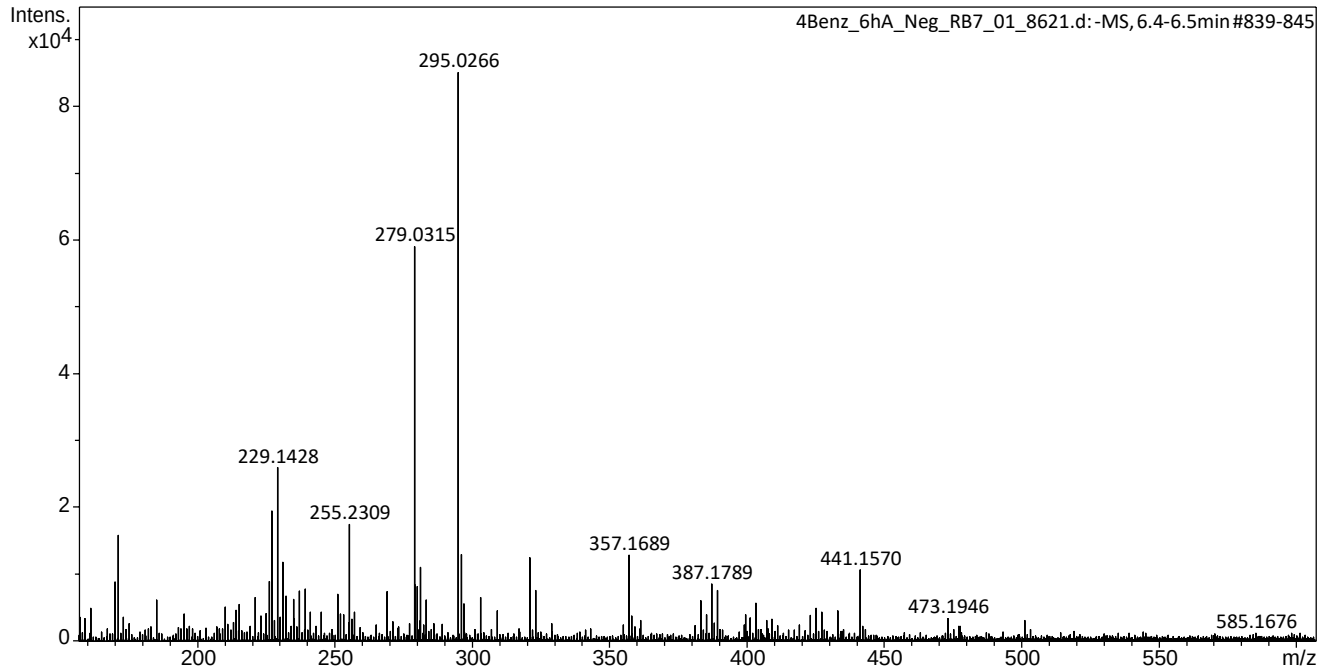
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

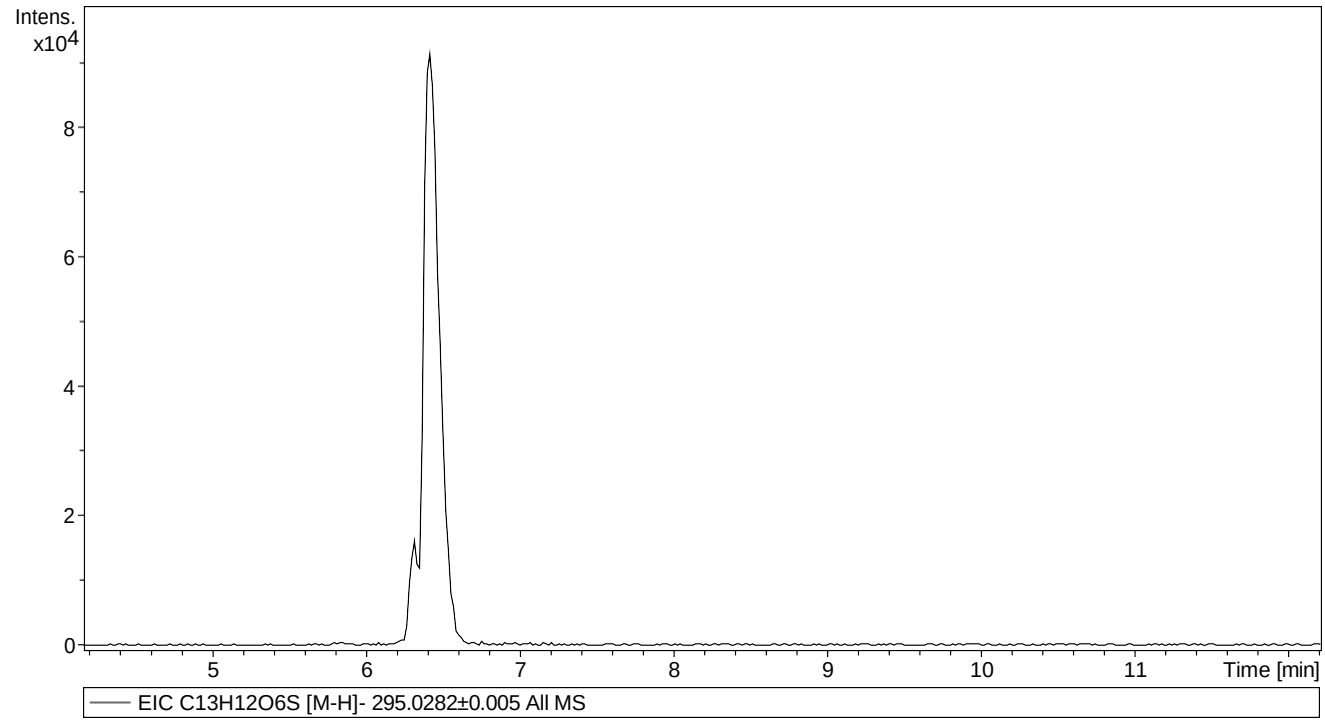
Operator BDAL@DE

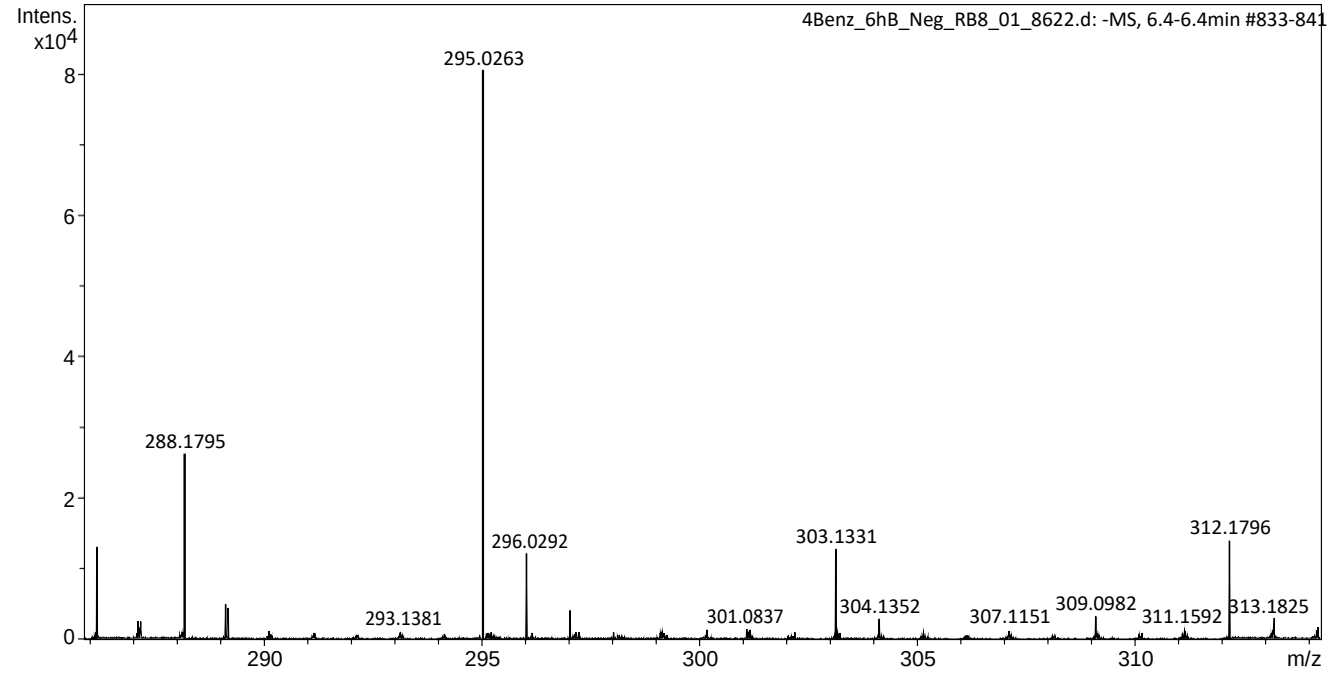
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





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Acquisition Date 06/12/2016 21:22:02

Operator BDAL@DE

Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
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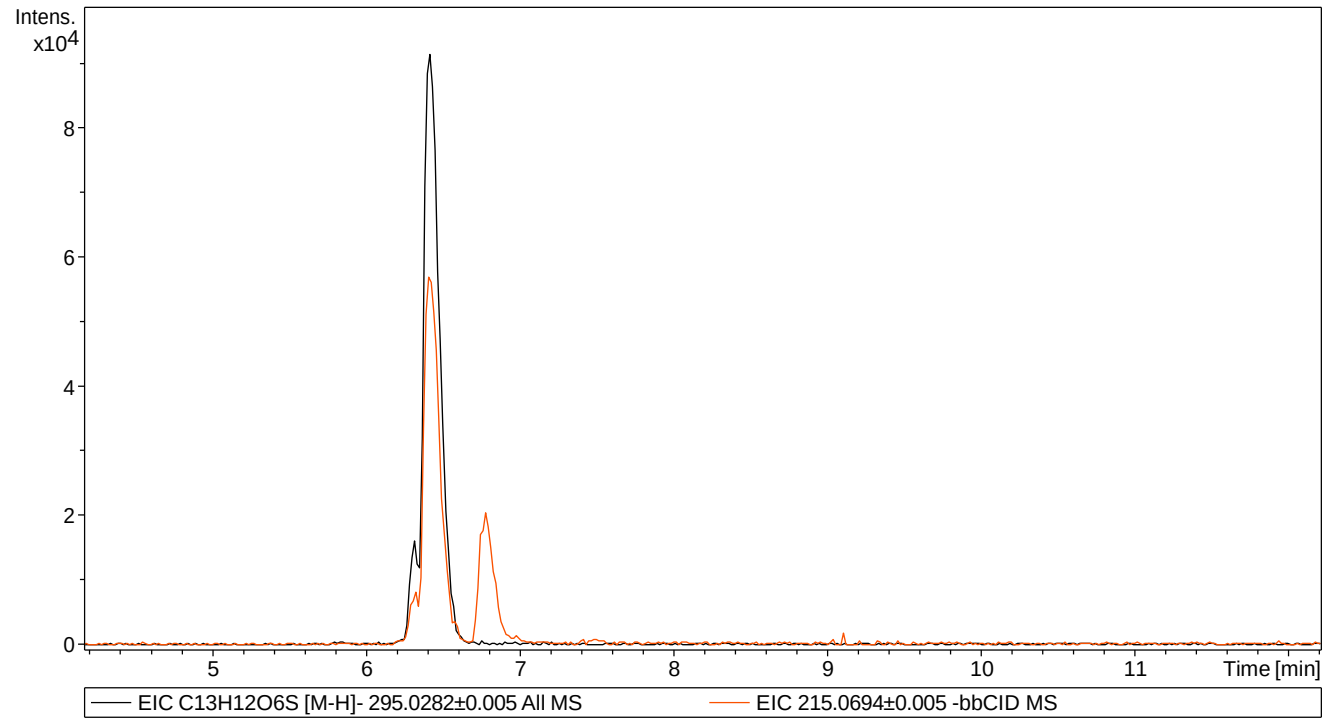
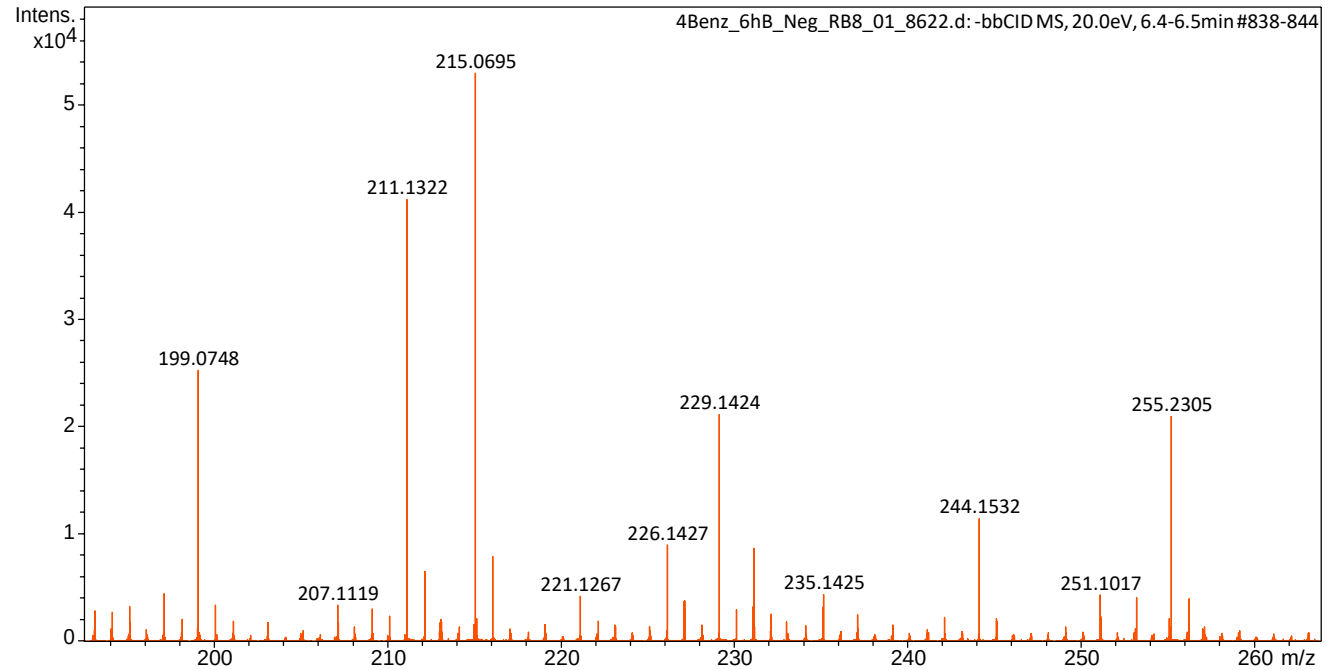
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:00:46

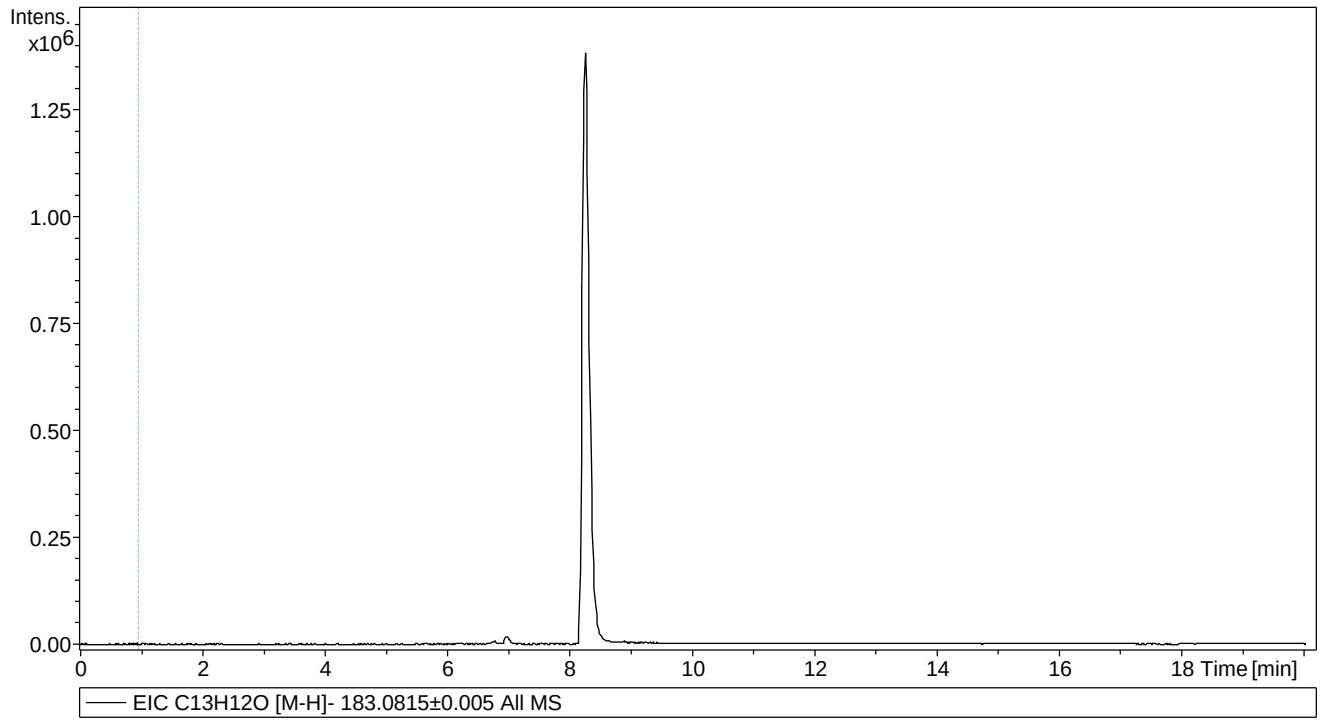
Operator BDAL@DE

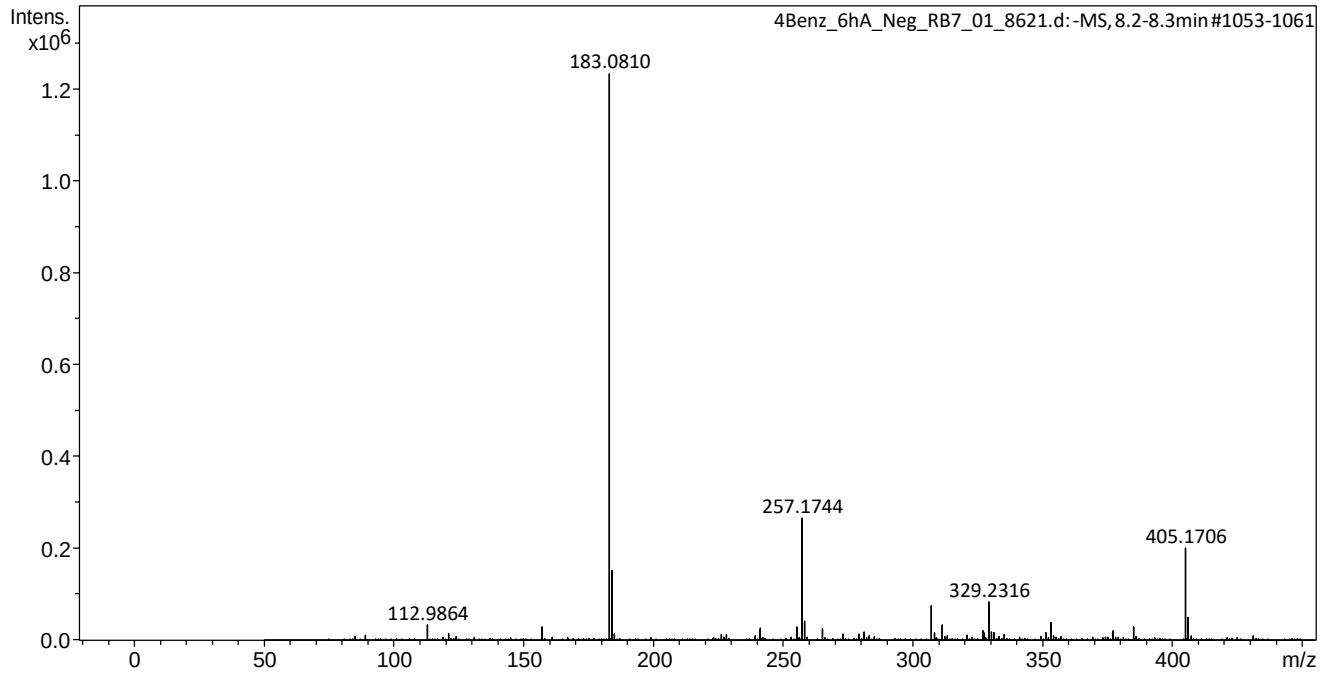
Sample Name 4Benz_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:22:02

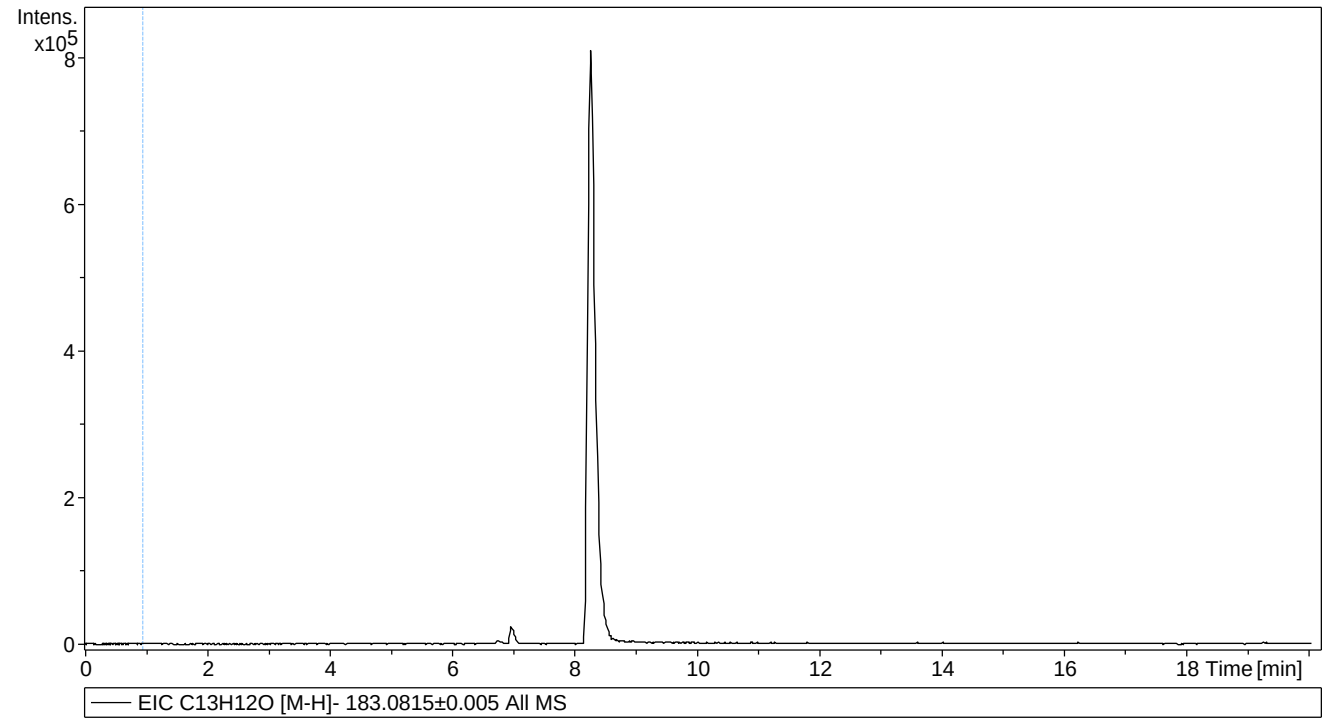
Operator BDAL@DE

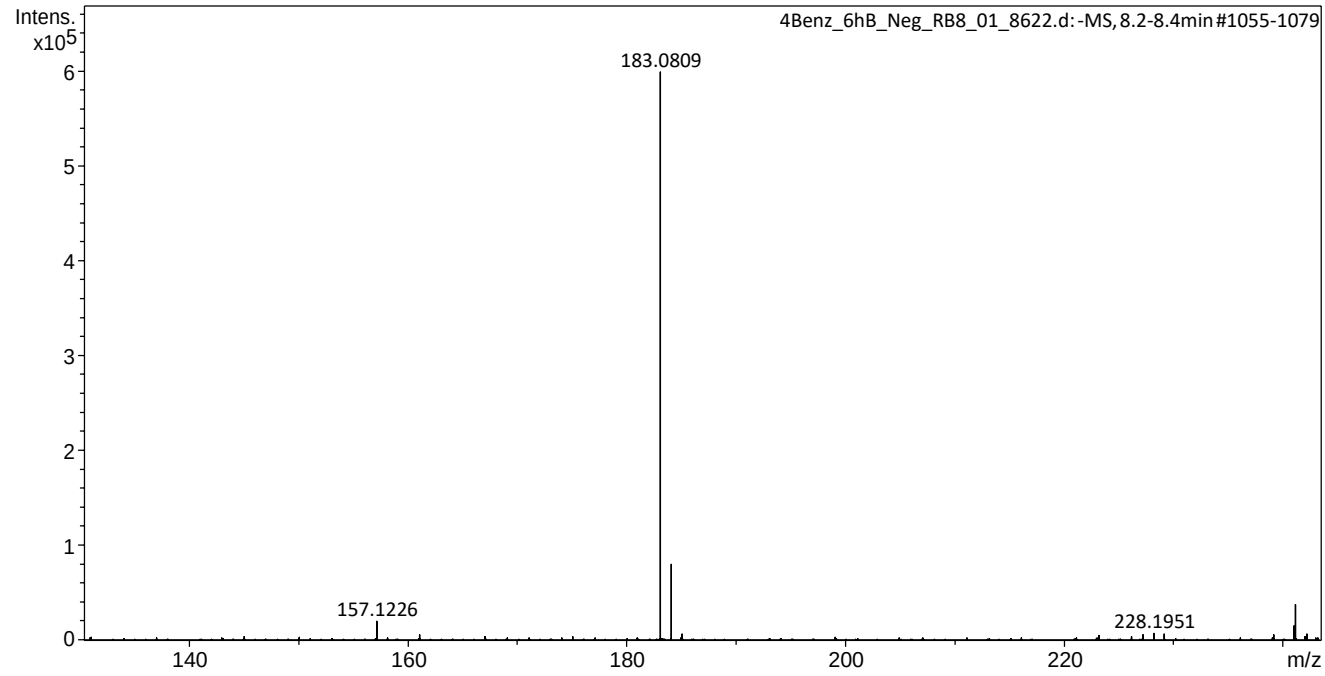
Sample Name 4Benz_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

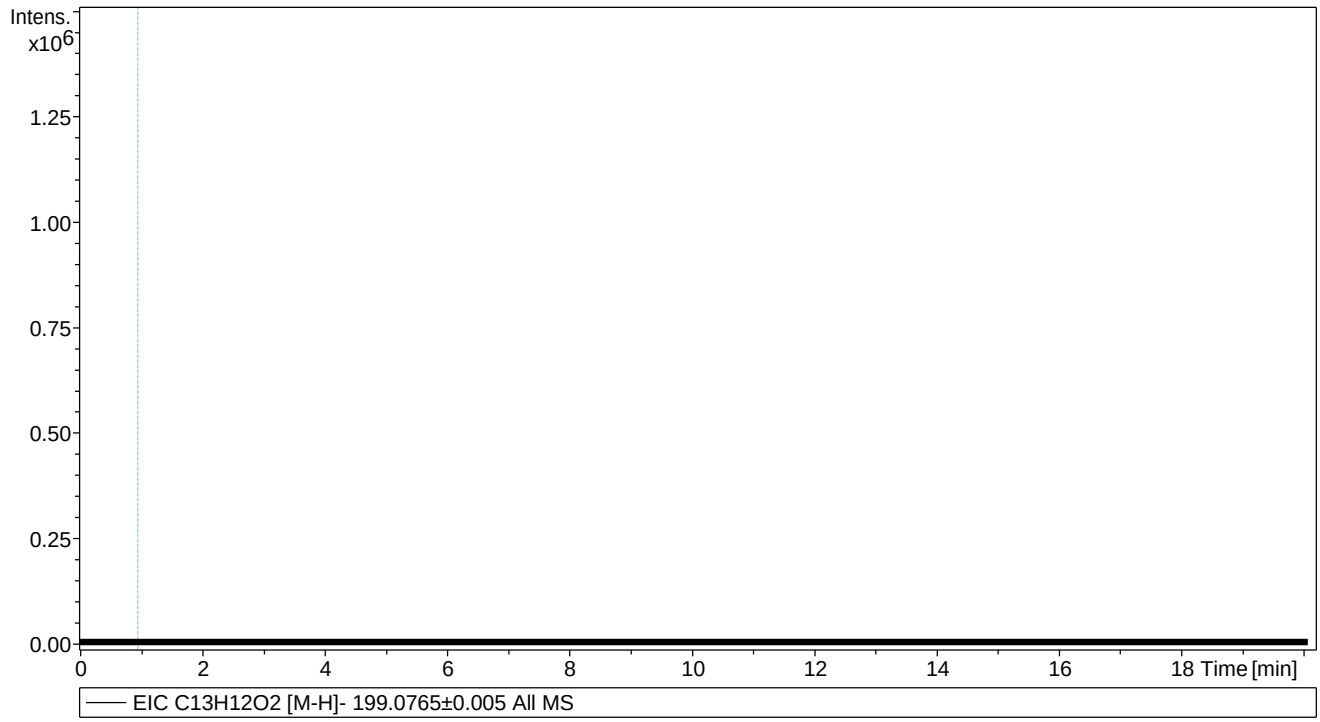
Operator BDAL@DE

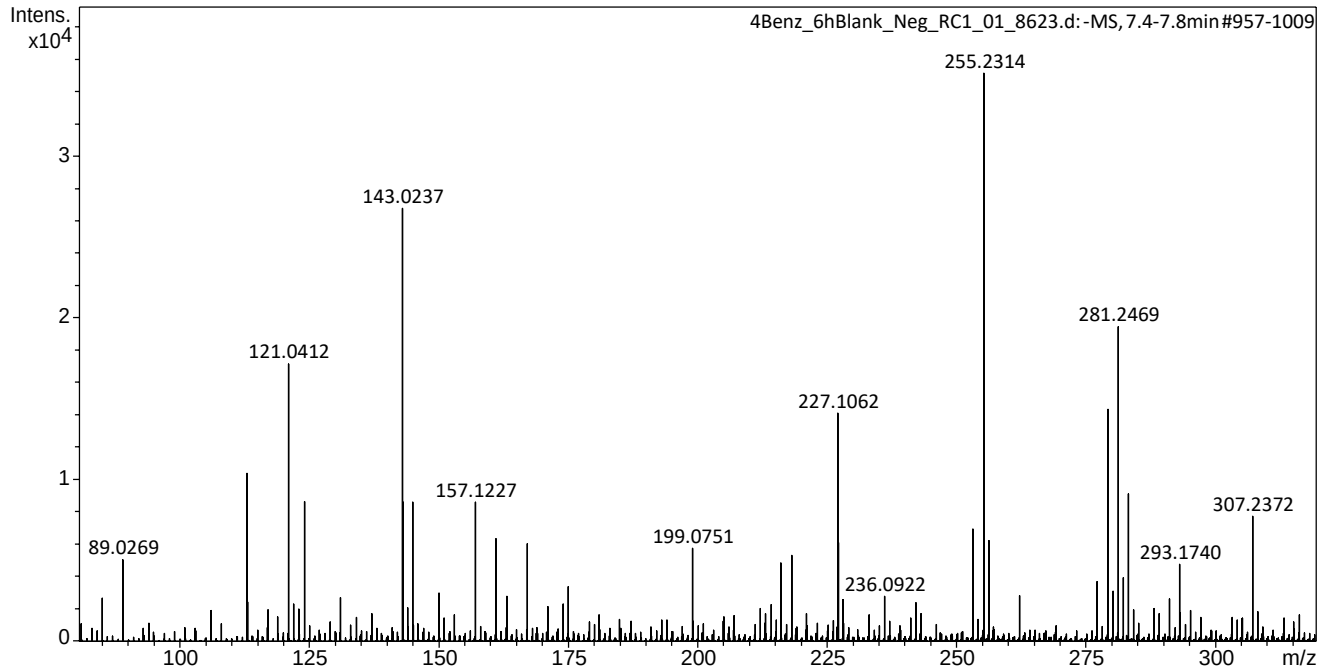
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

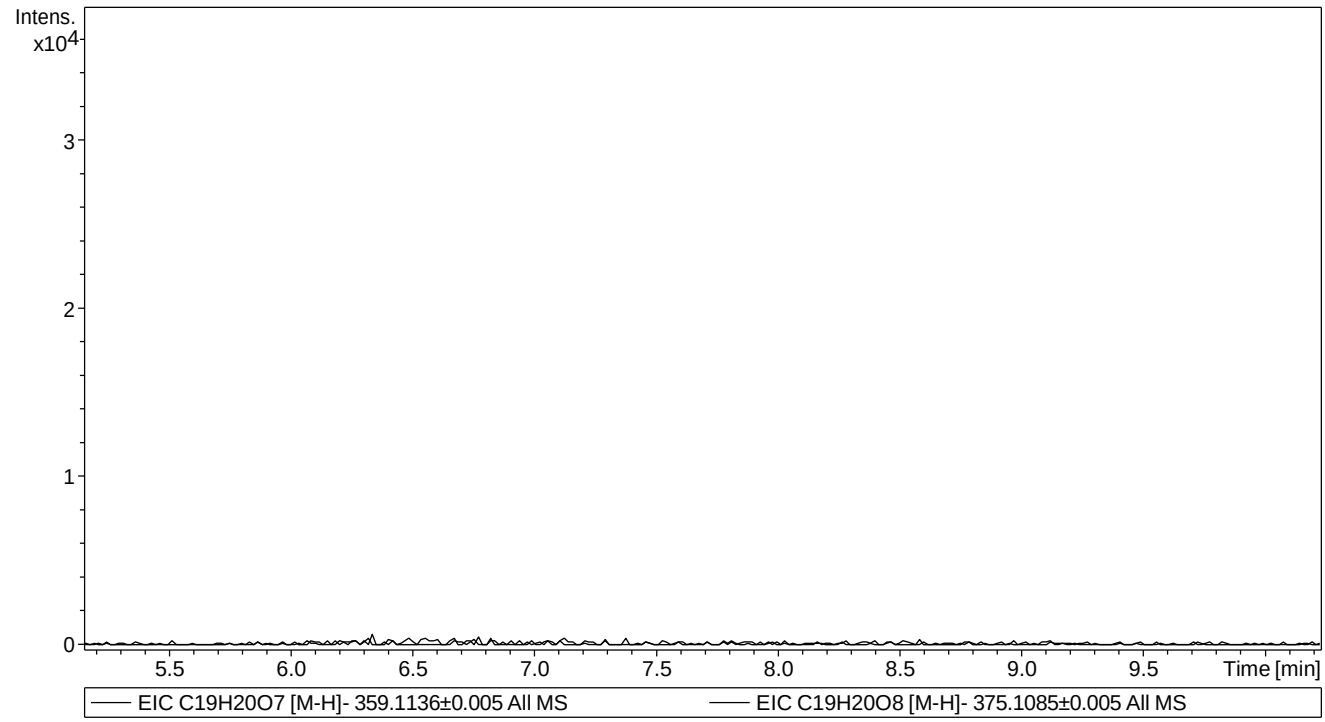
Operator BDAL@DE

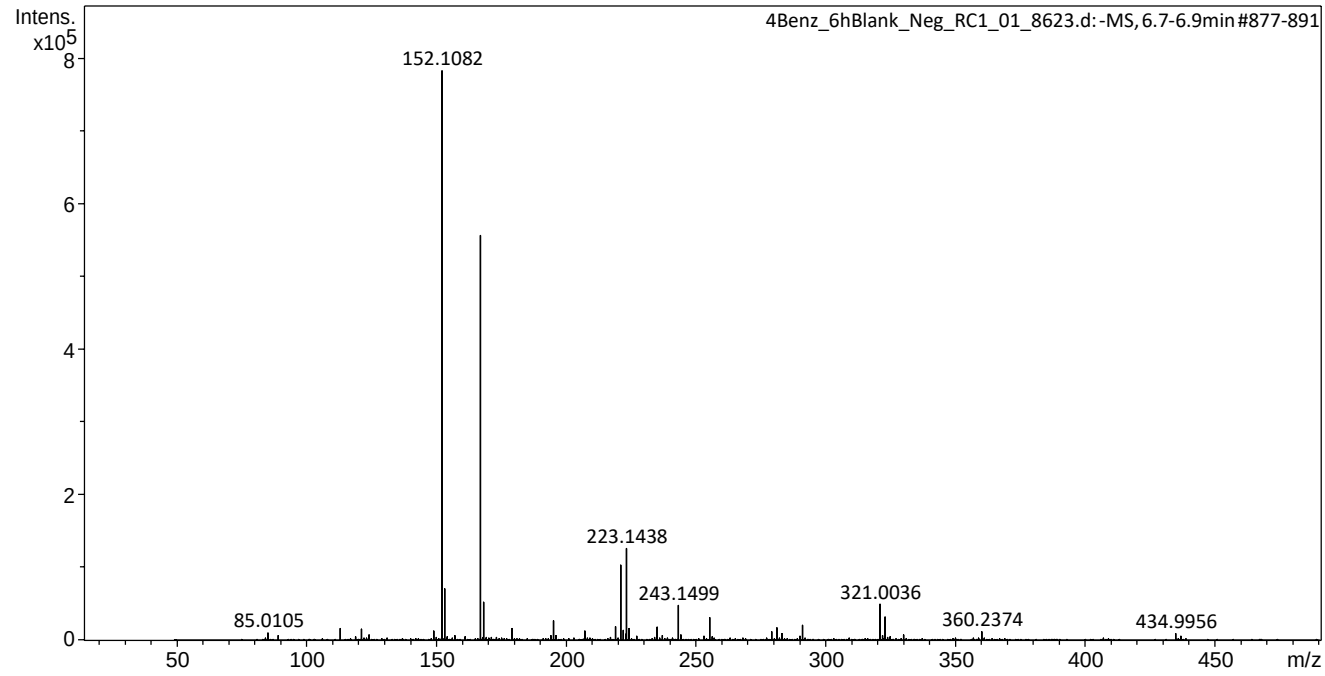
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

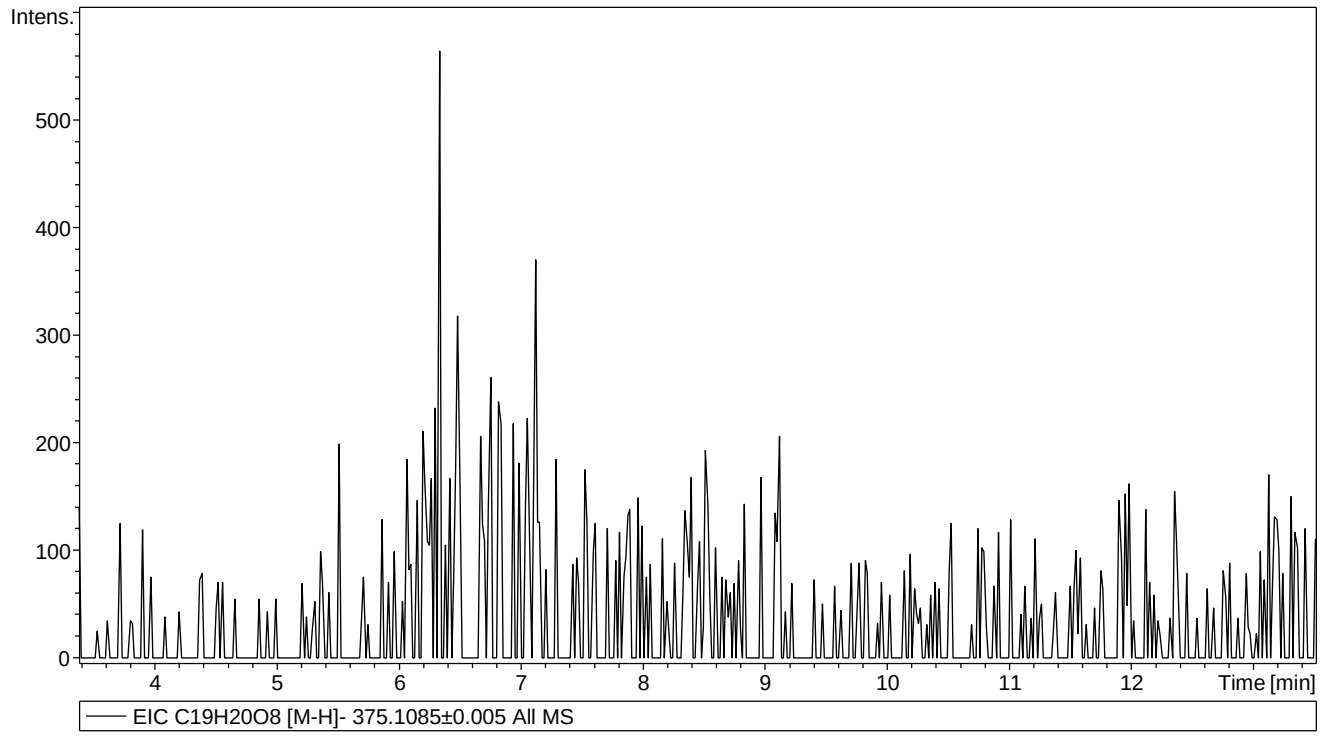
Operator BDAL@DE

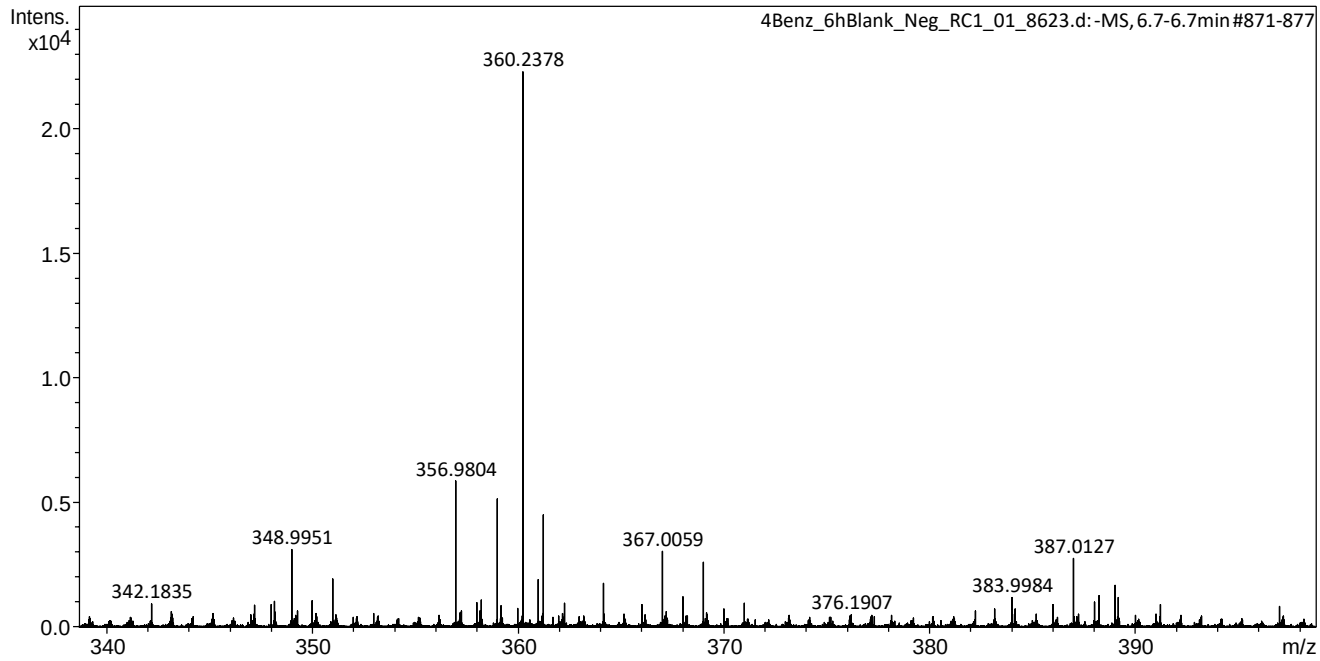
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





4Benz_6hBlank_Neg_RC1_01_8623.d

Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

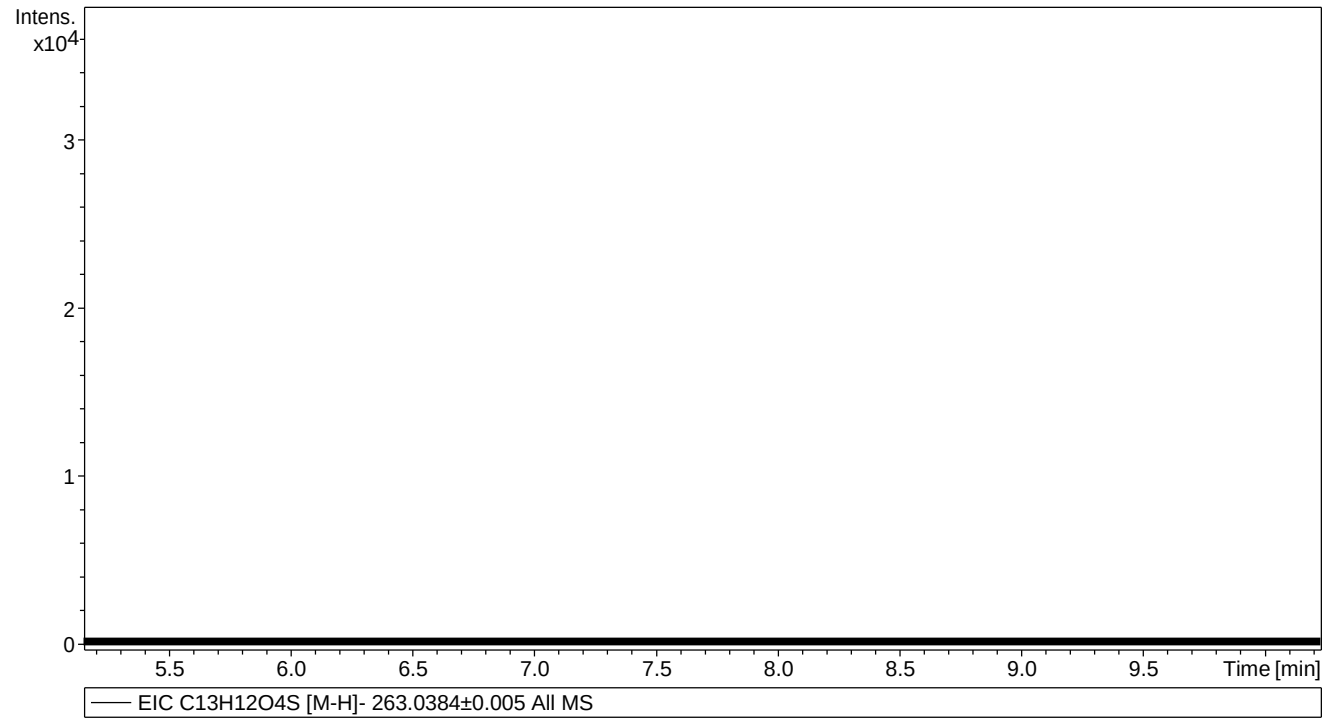
Operator BDAL@DE

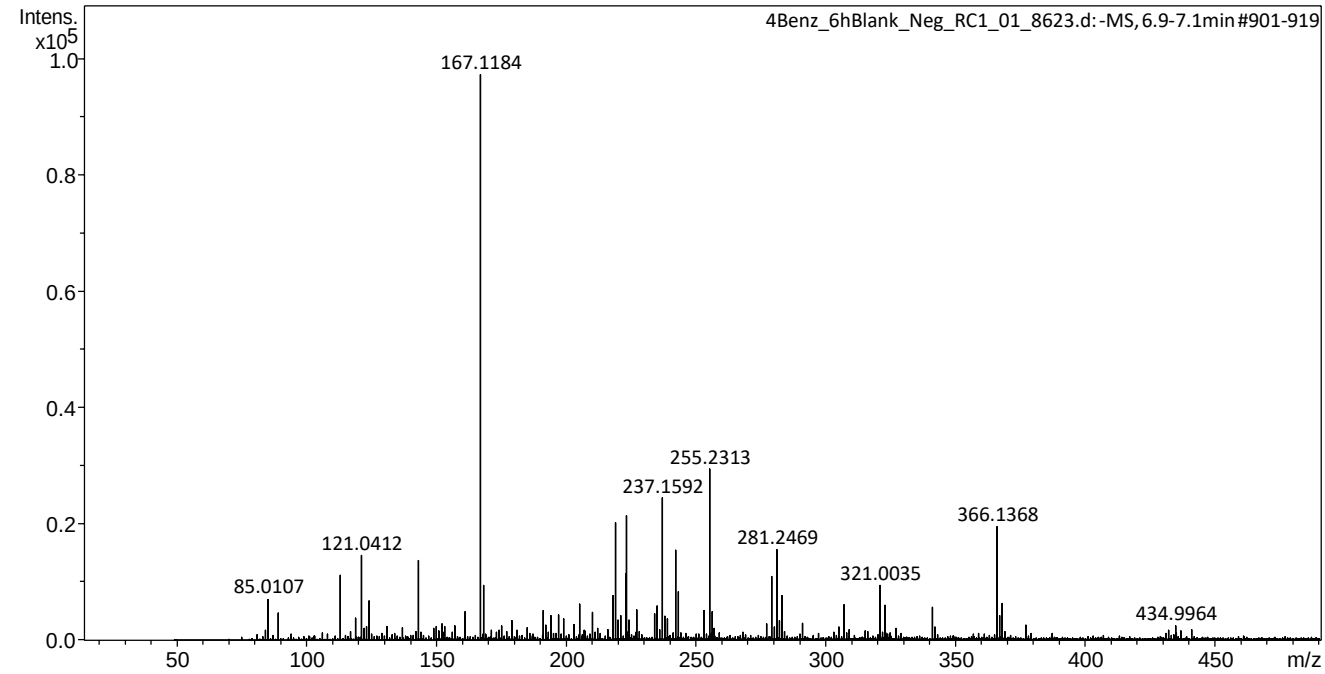
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





4Benz_6hBlank_Neg_RC1_01_8623.d

Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

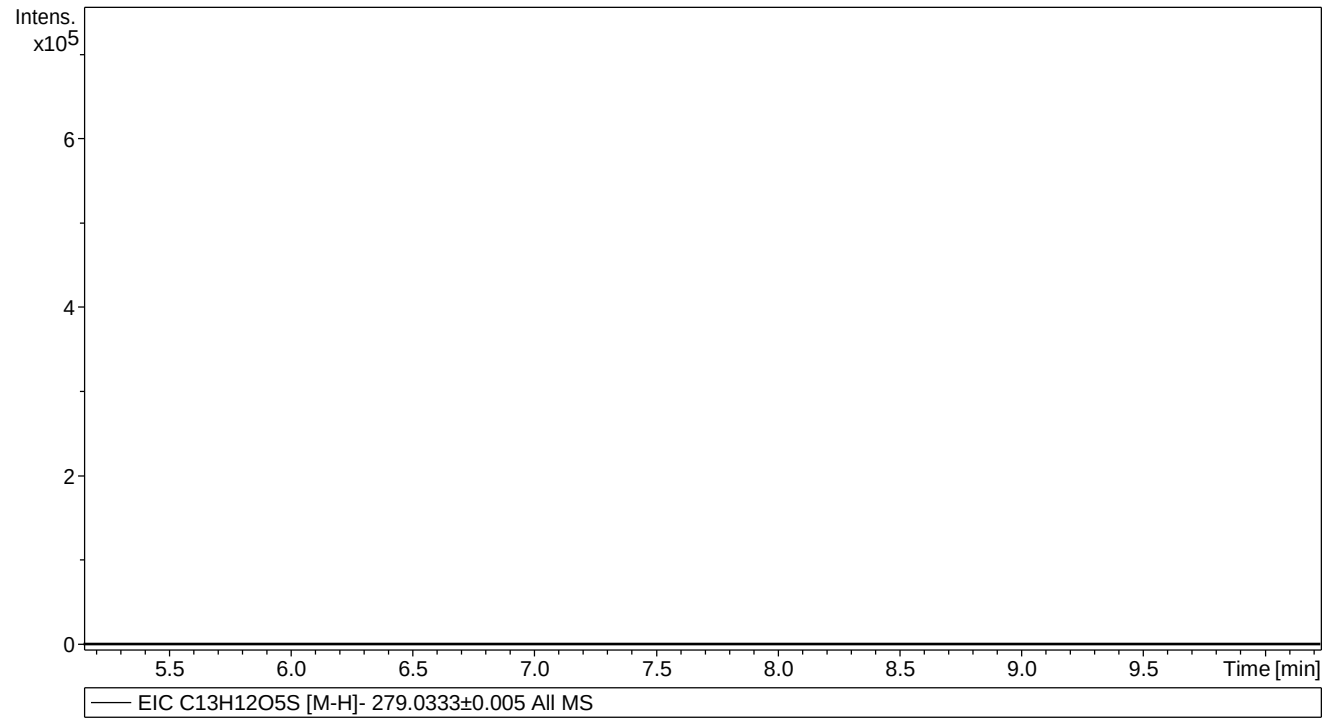
Operator BDAL@DE

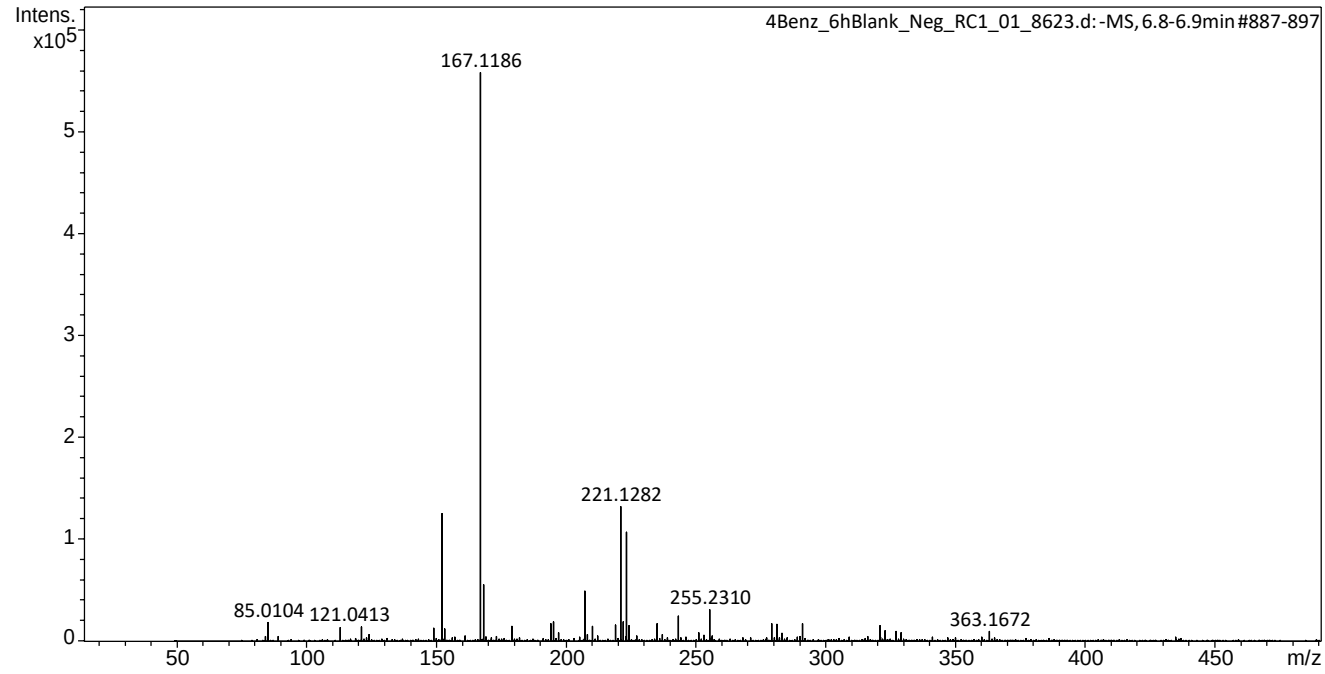
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 06/12/2016 21:43:17

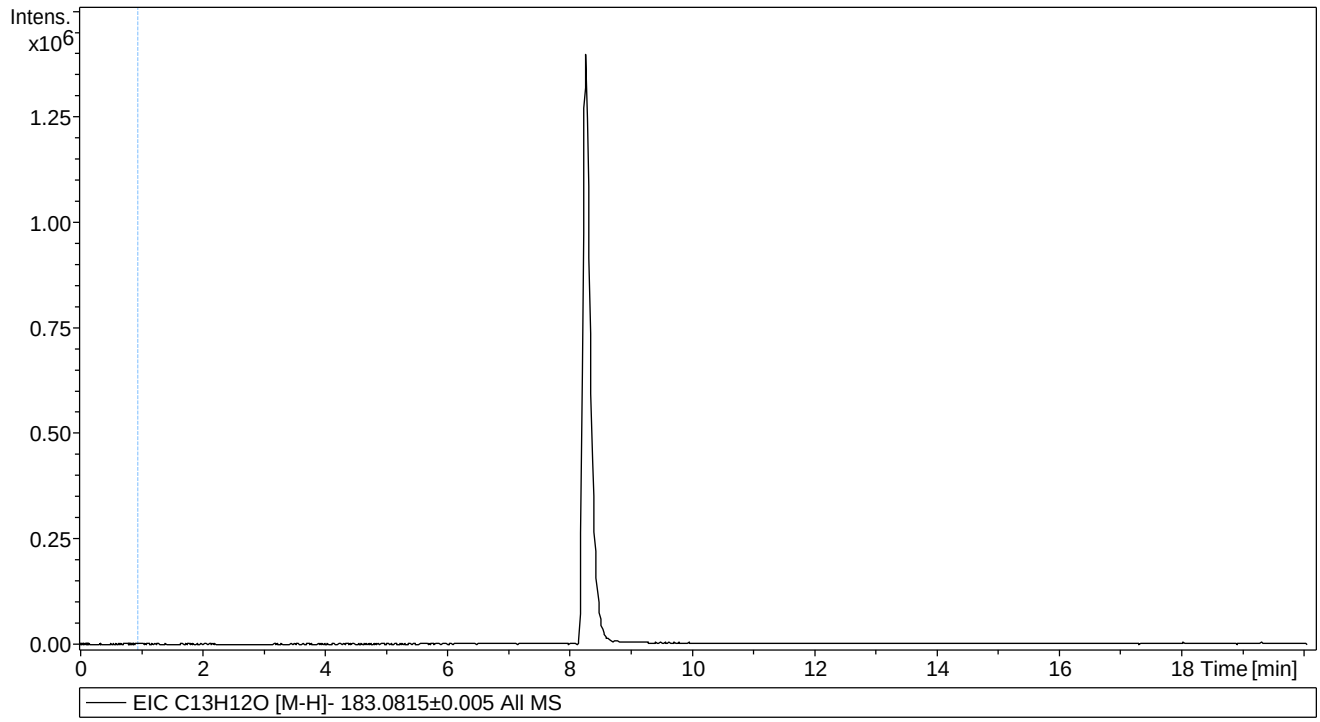
Operator BDAL@DE

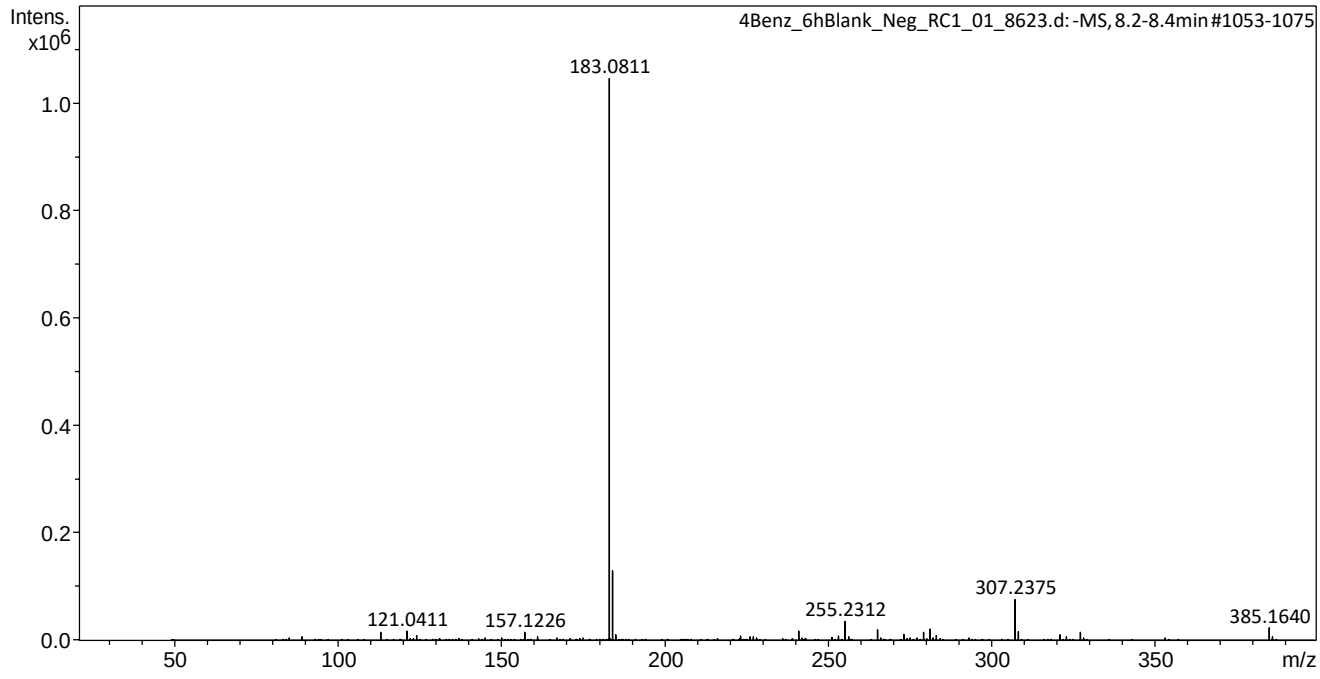
Sample Name 4Benz_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 02:15:51

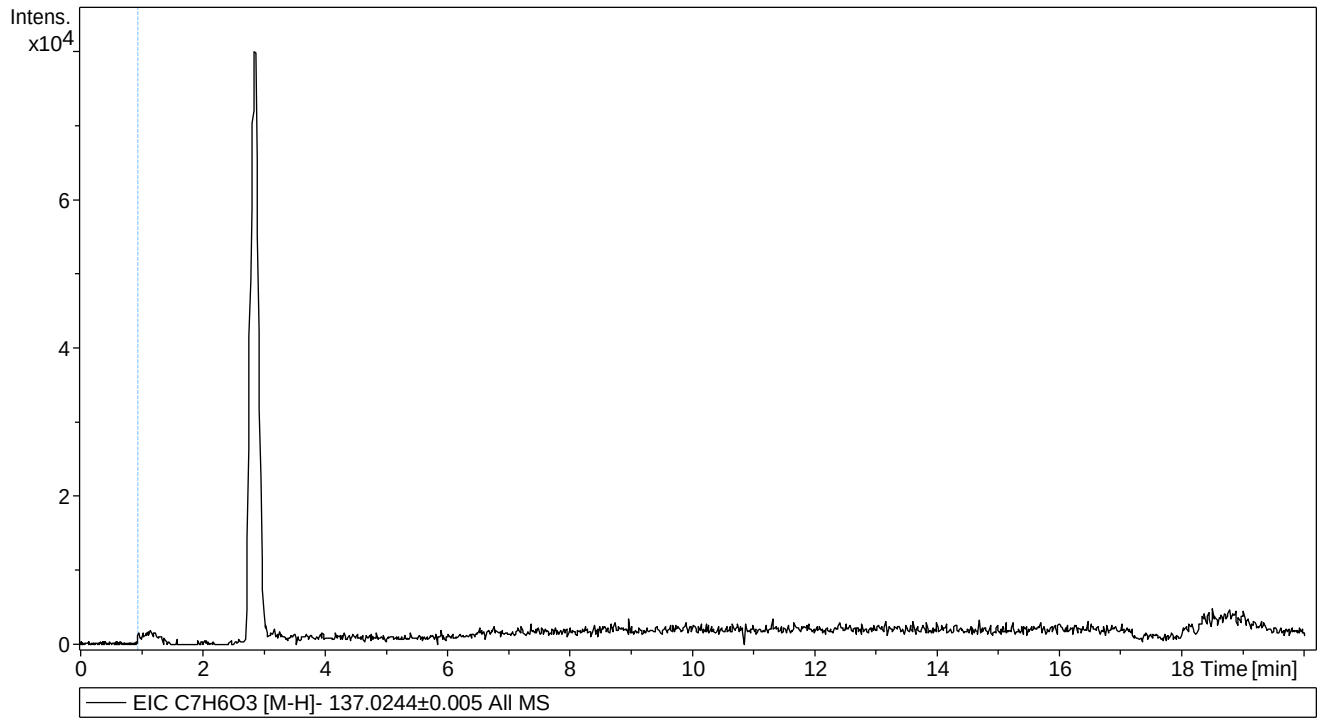
Operator BDAL@DE

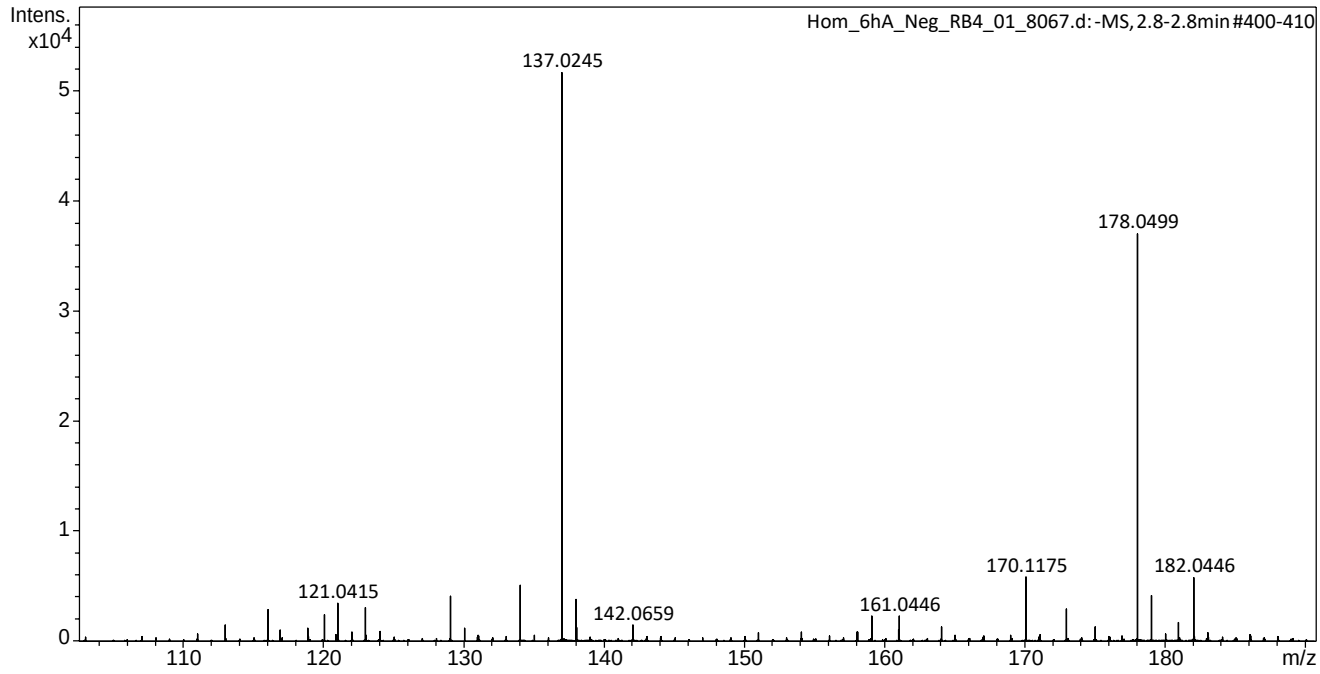
Sample Name Hom_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Hom_6hA_Neg_RB4_01_8067.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 02:15:51

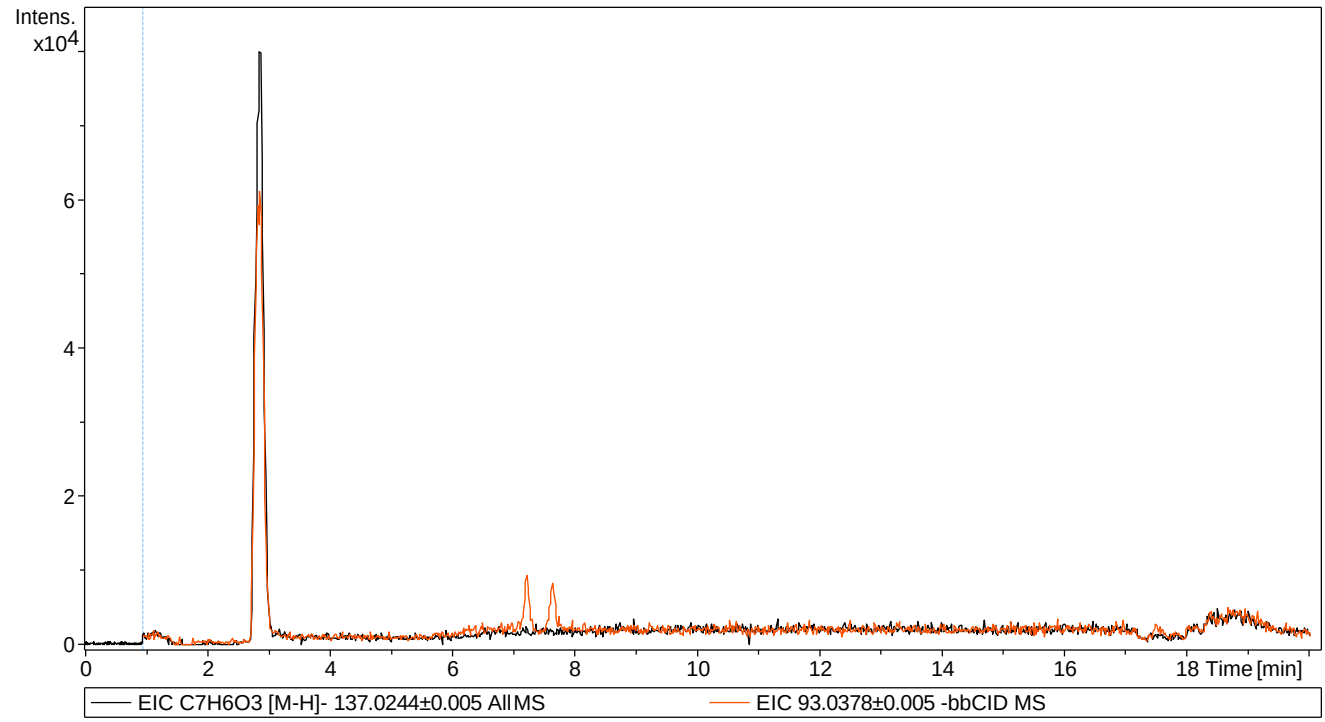
Operator BDAL@DE

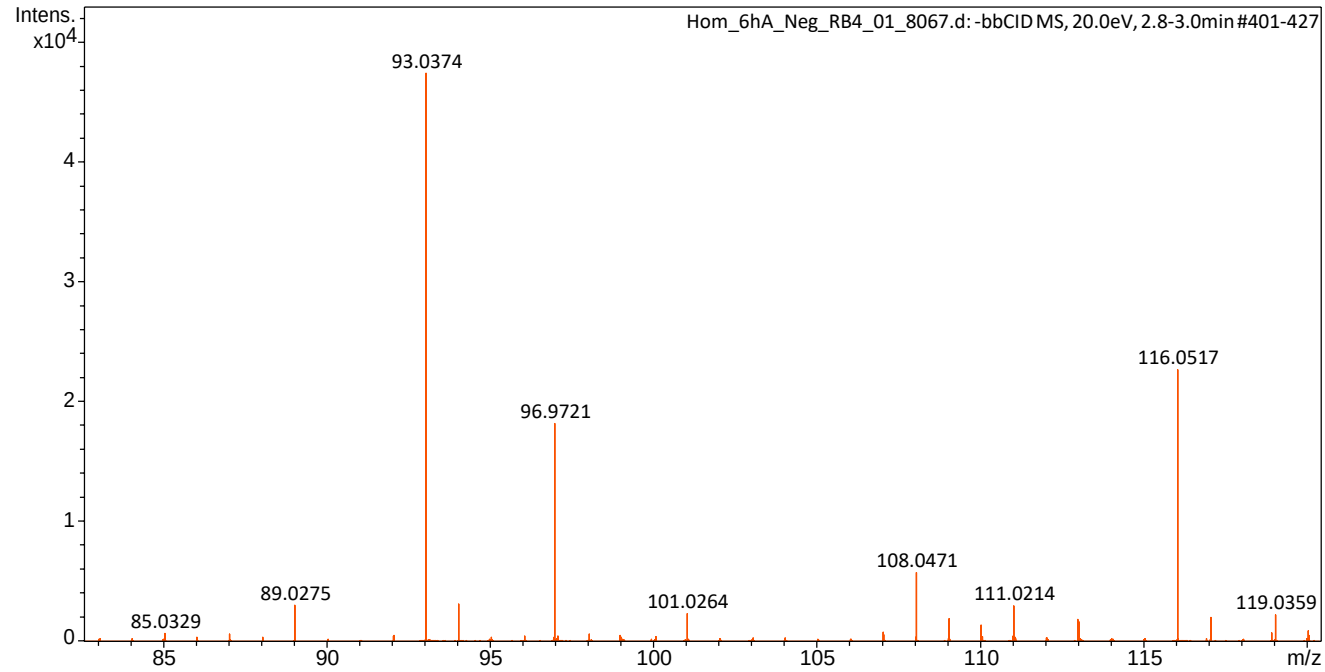
Sample Name Hom_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Hom_6hA_Neg_RB4_01_8067.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 02:37:06

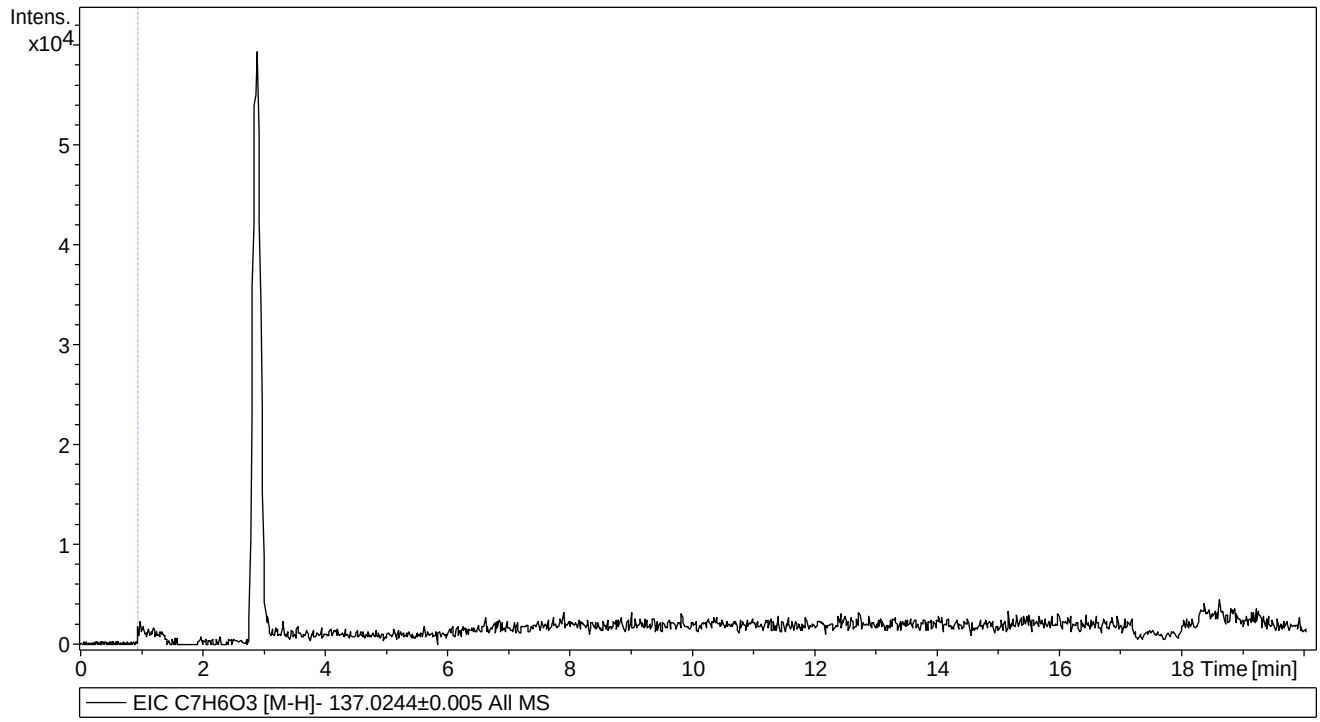
Operator BDAL@DE

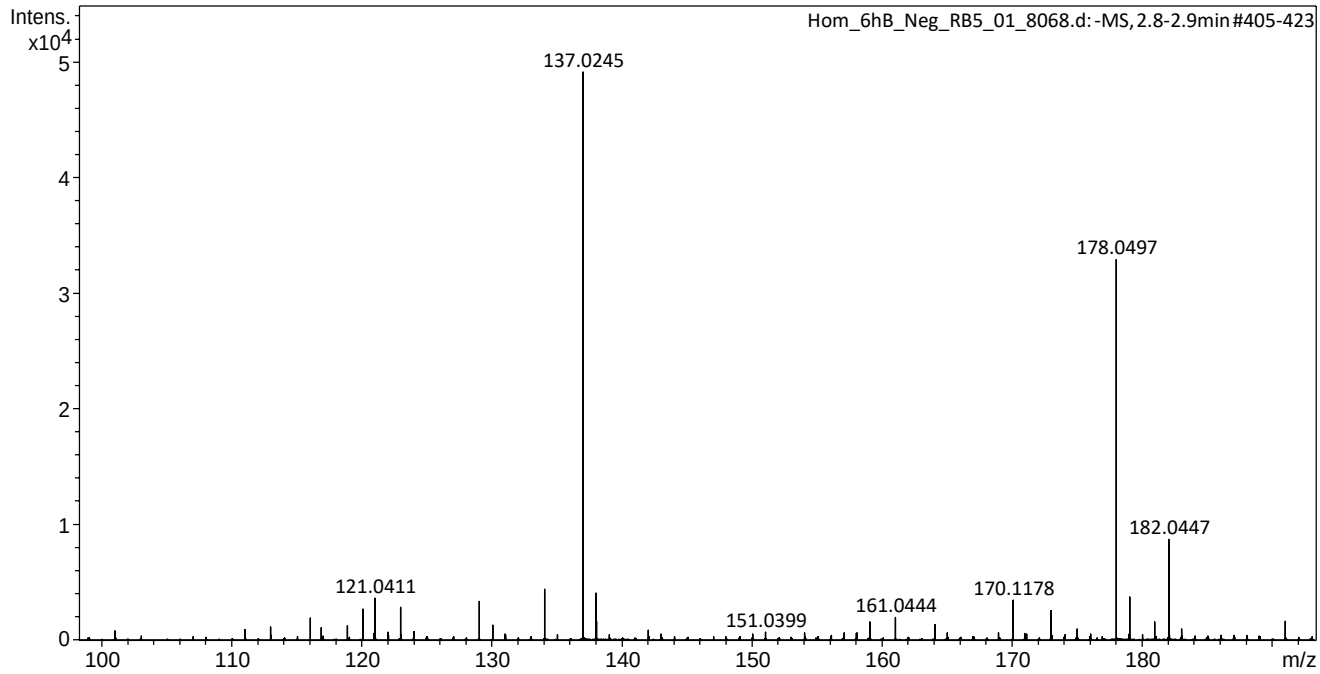
Sample Name Hom_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 02:37:06

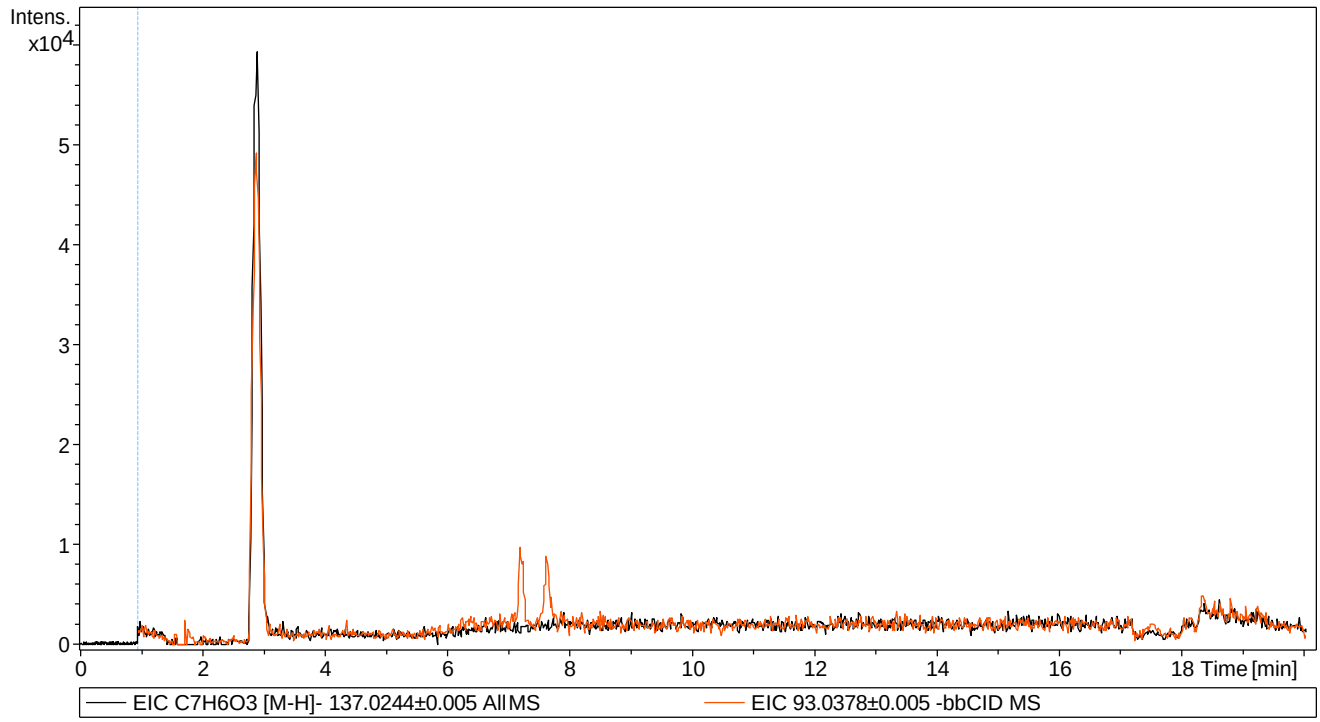
Operator BDAL@DE

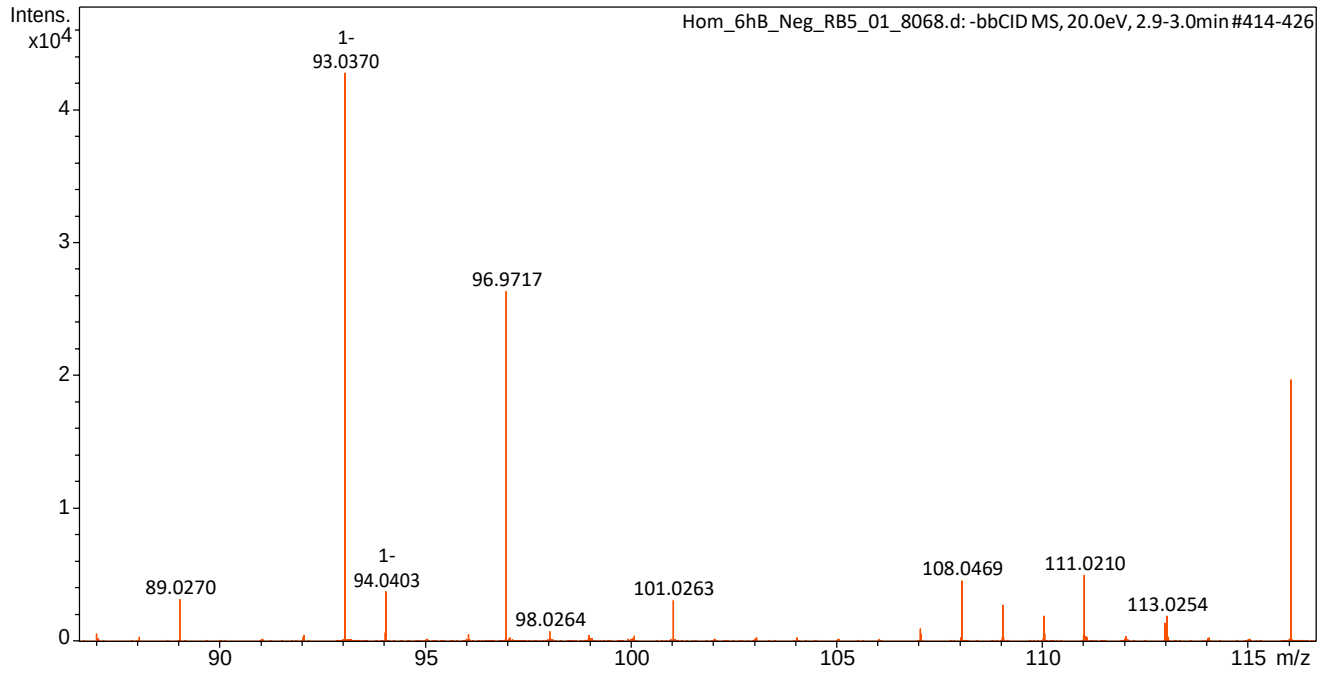
Sample Name Hom_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 02:58:22

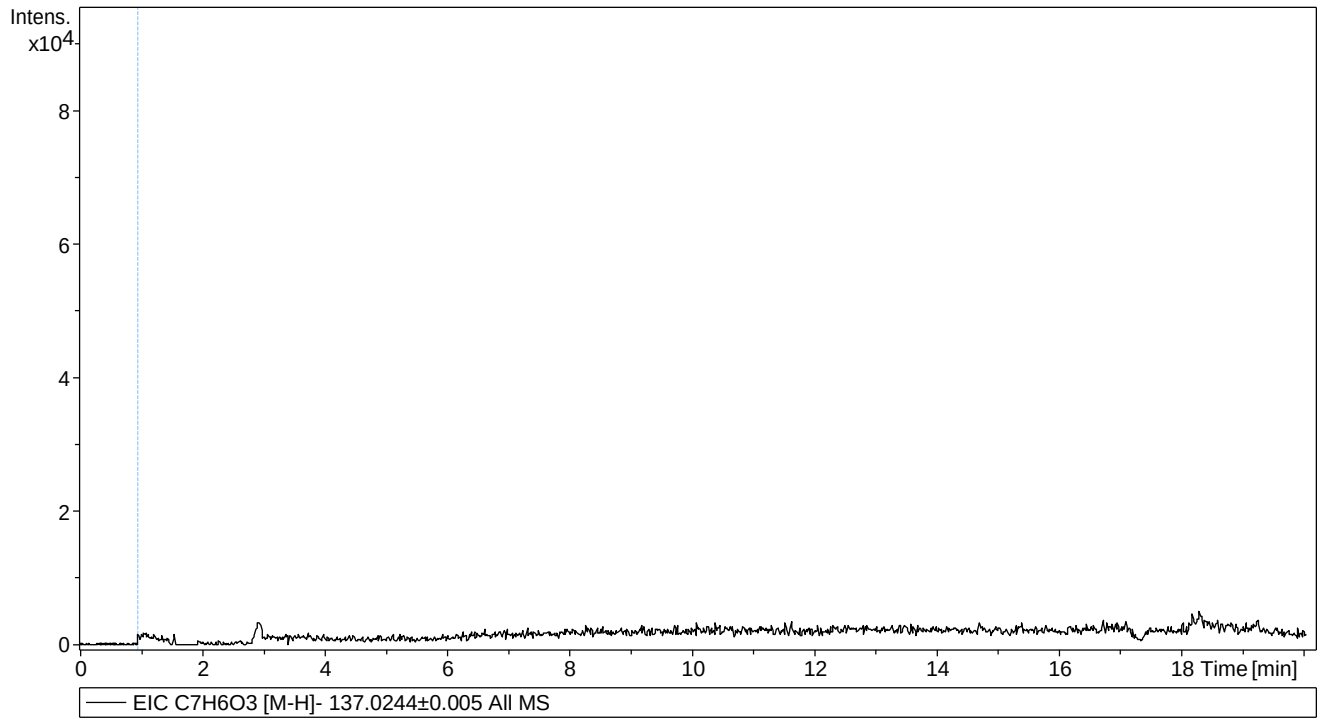
Operator BDAL@DE

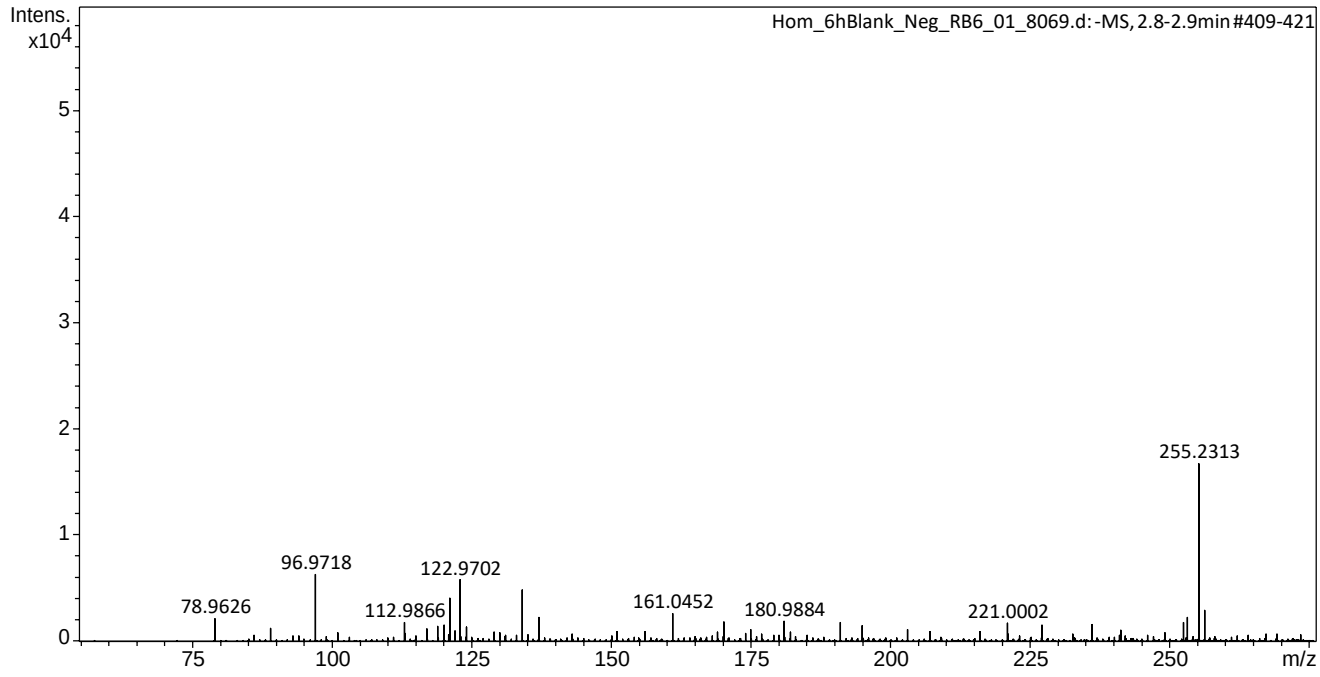
Sample Name Hom_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Hom_6hBlank_Neg_RB6_01_8069.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 03:19:39

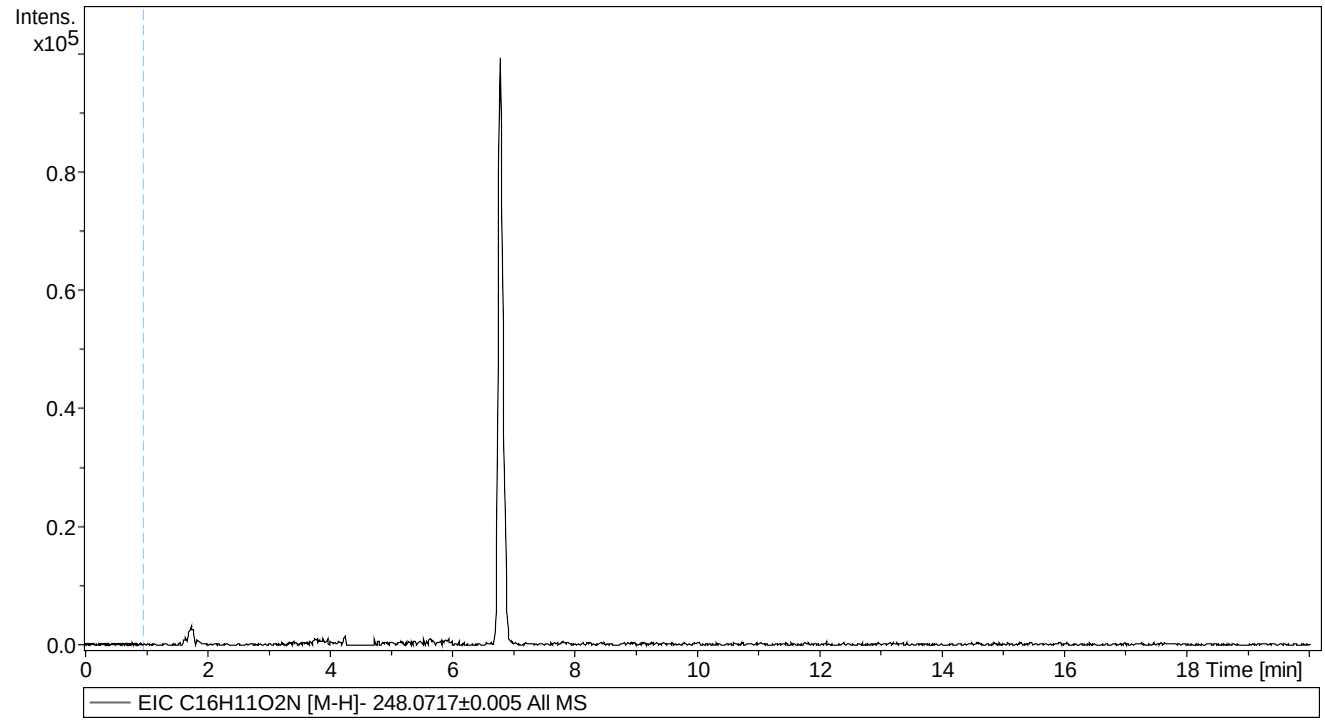
Operator BDAL@DE

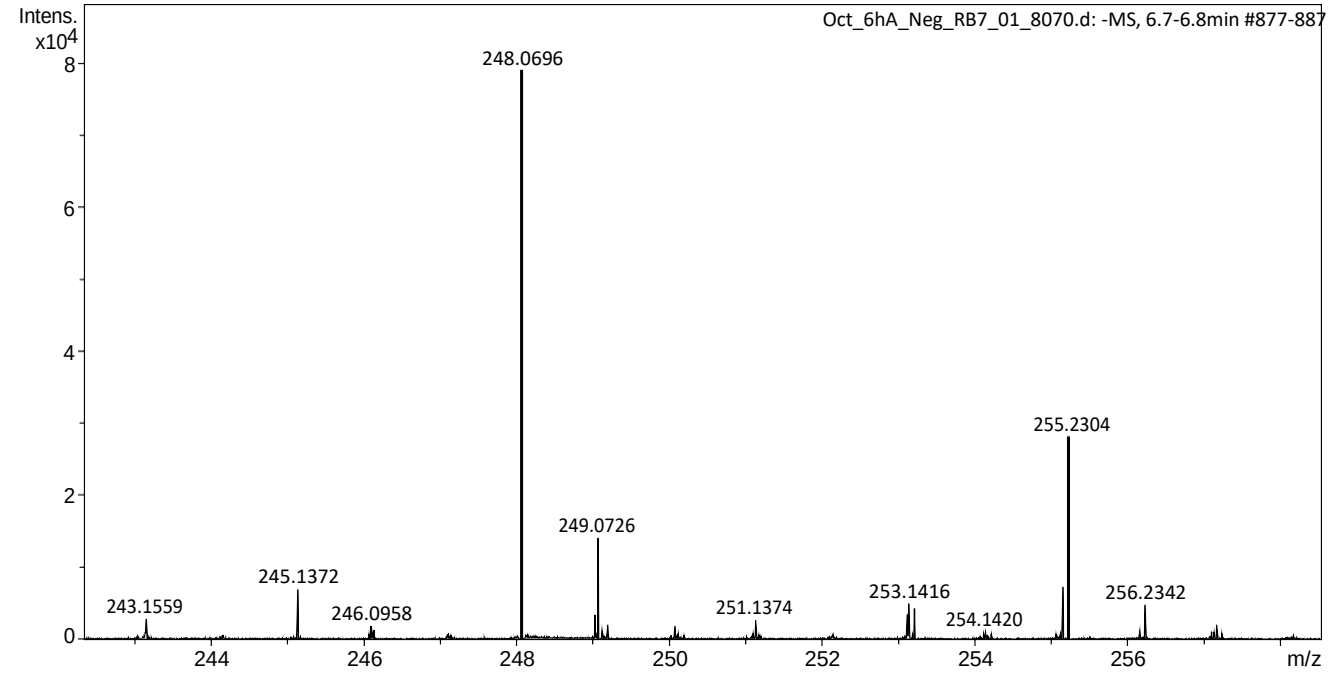
Sample Name Oct_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 03:19:39

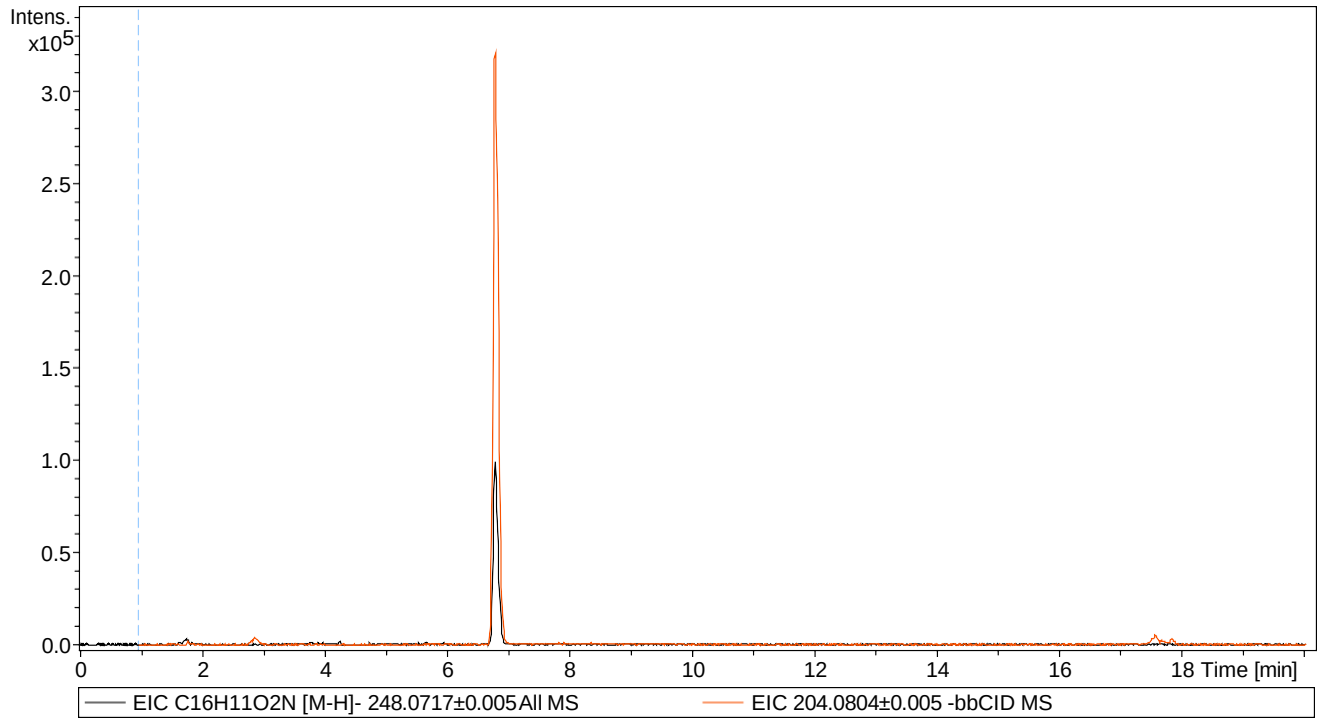
Operator BDAL@DE

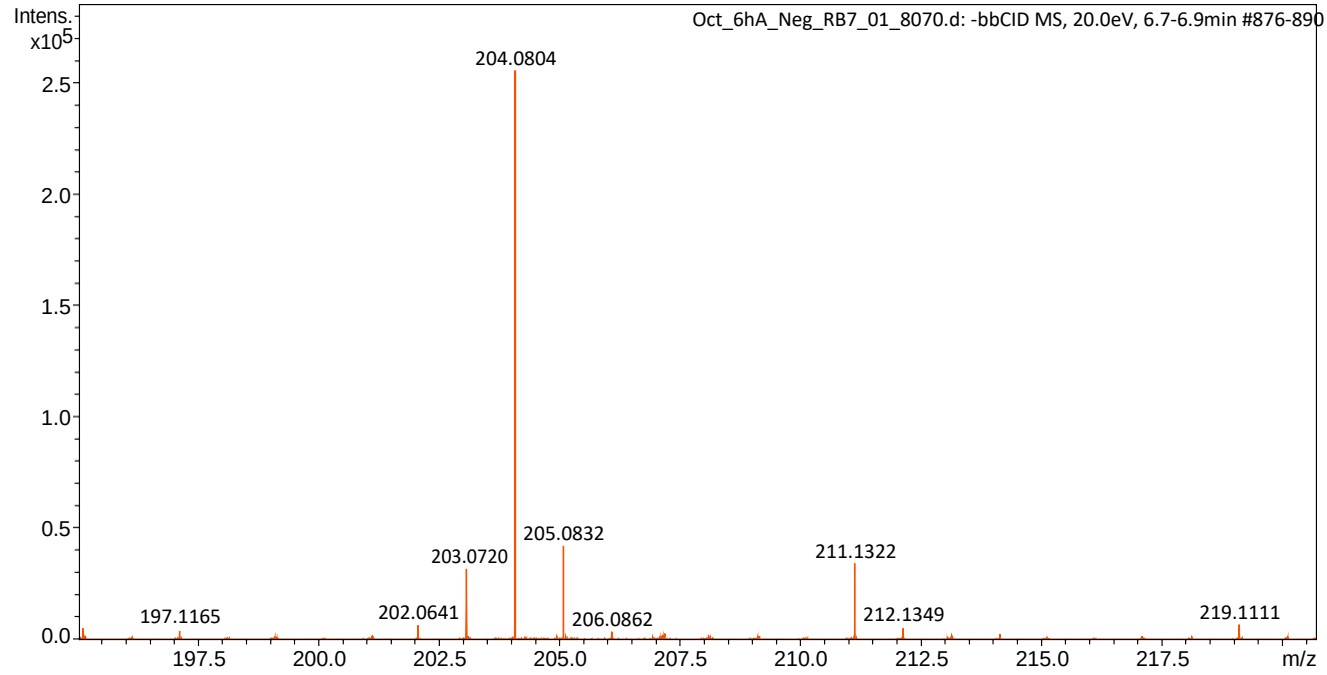
Sample Name Oct_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 03:40:56

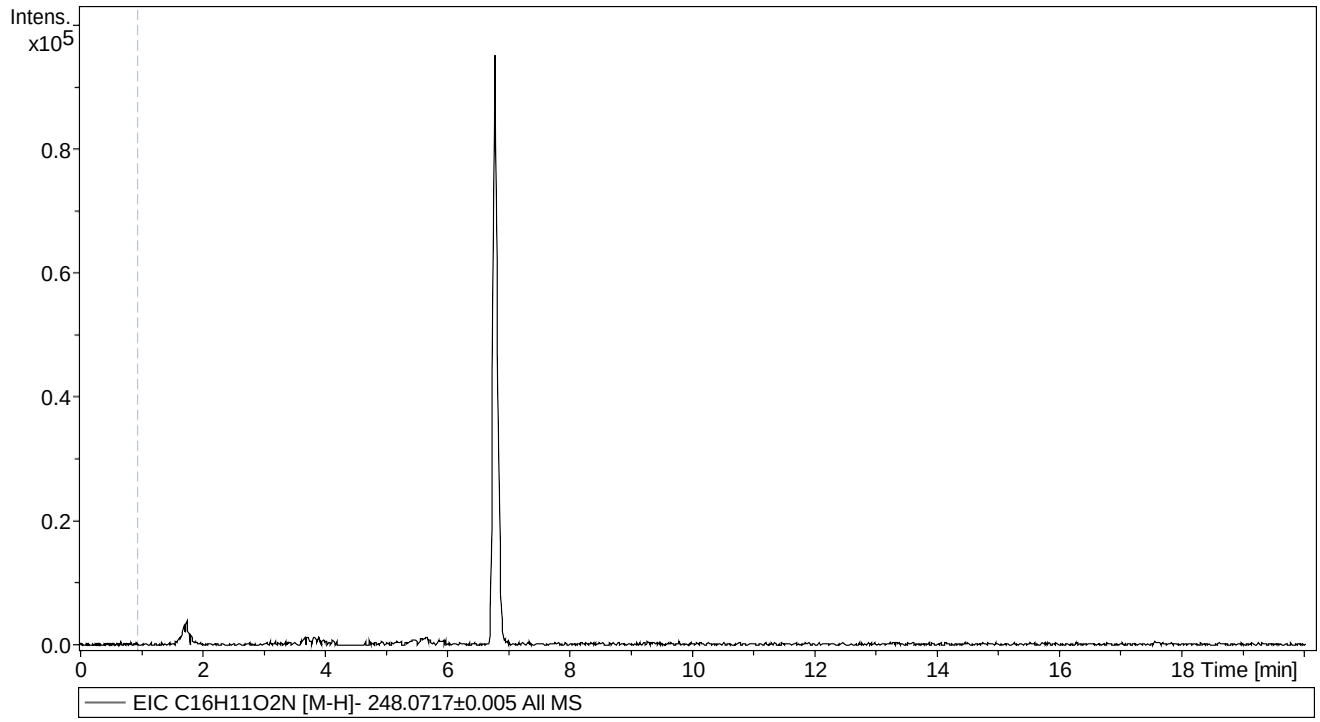
Operator BDAL@DE

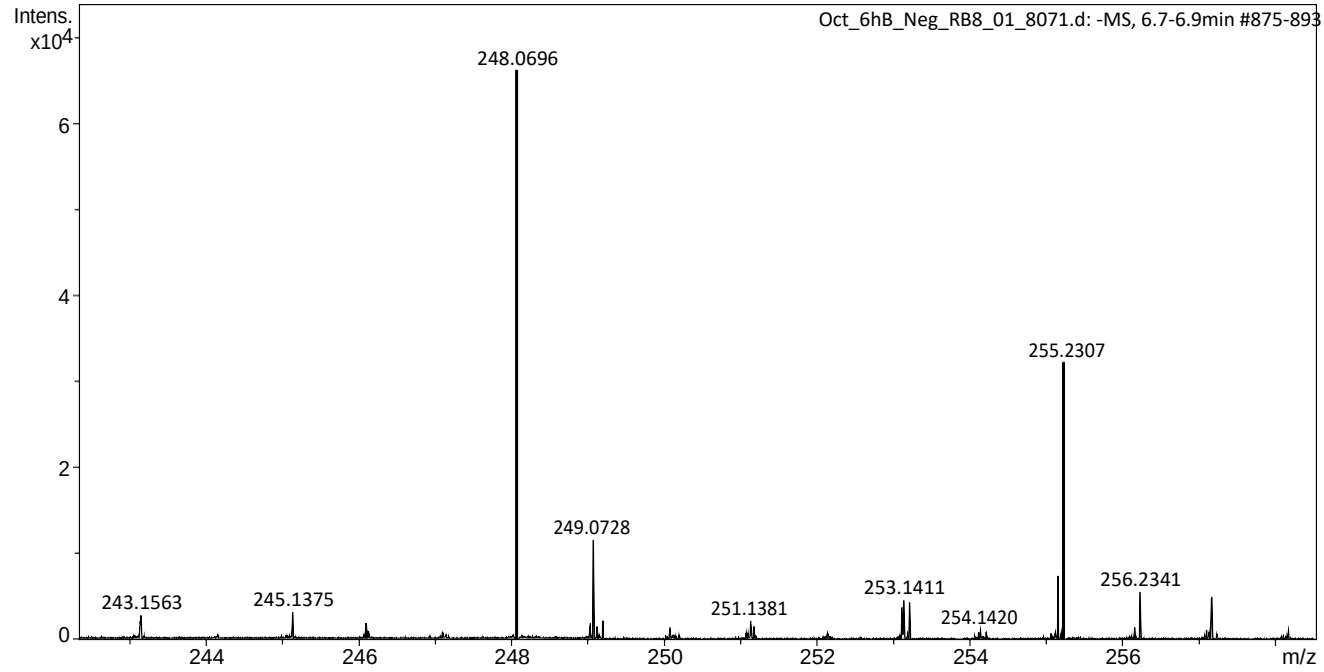
Sample Name Oct_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Oct_6hB_Neg_RB8_01_8071.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 03:40:56

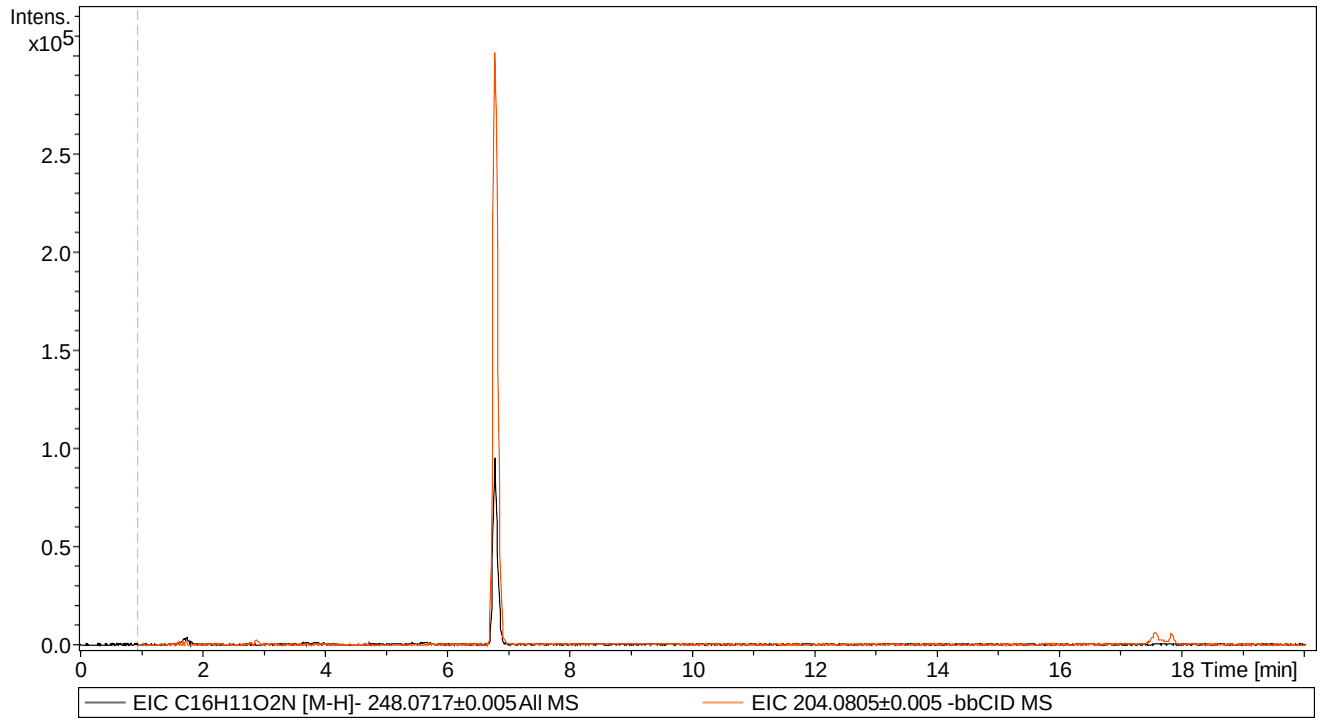
Operator BDAL@DE

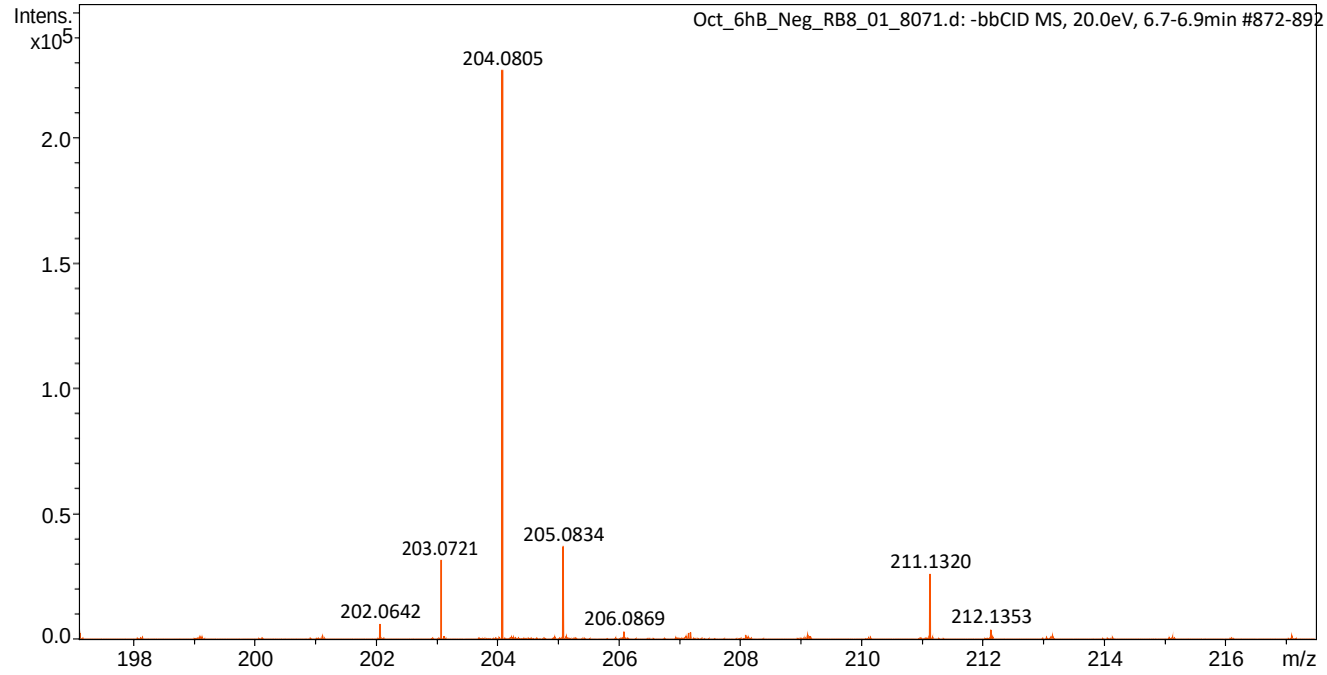
Sample Name Oct_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 04:2:10

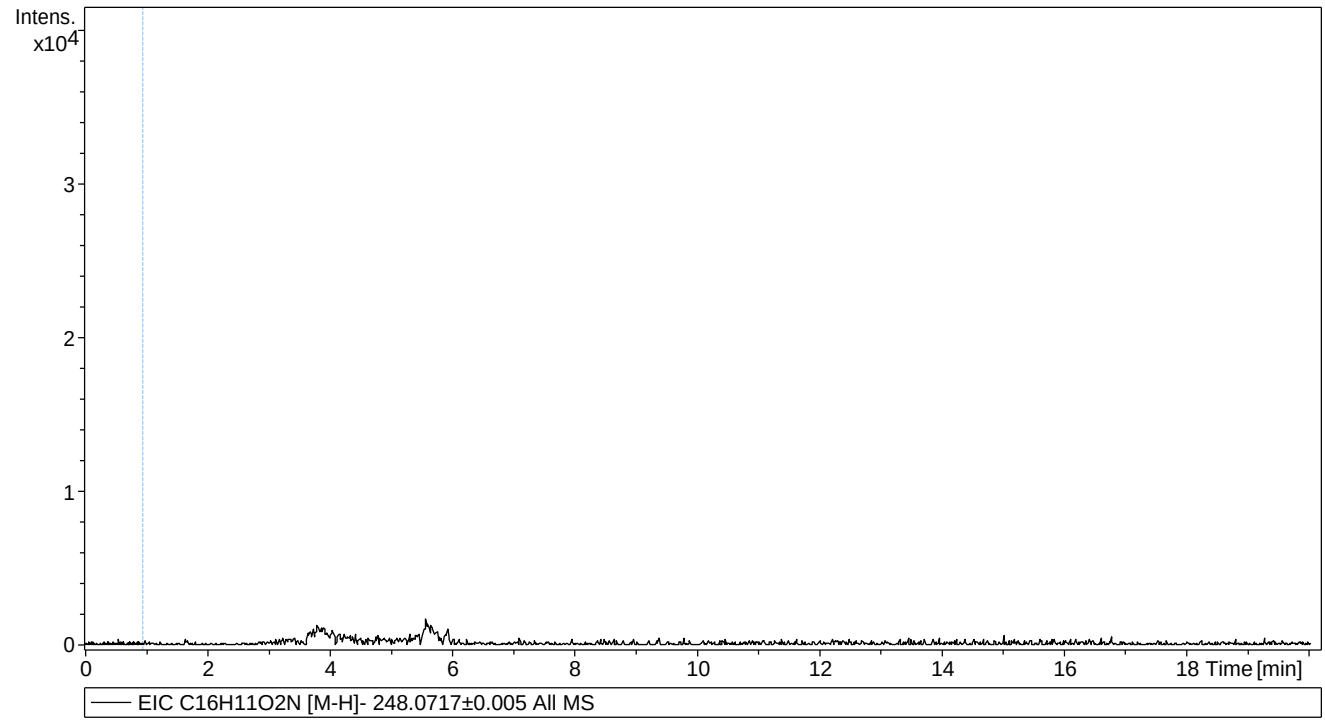
Operator BDAL@DE

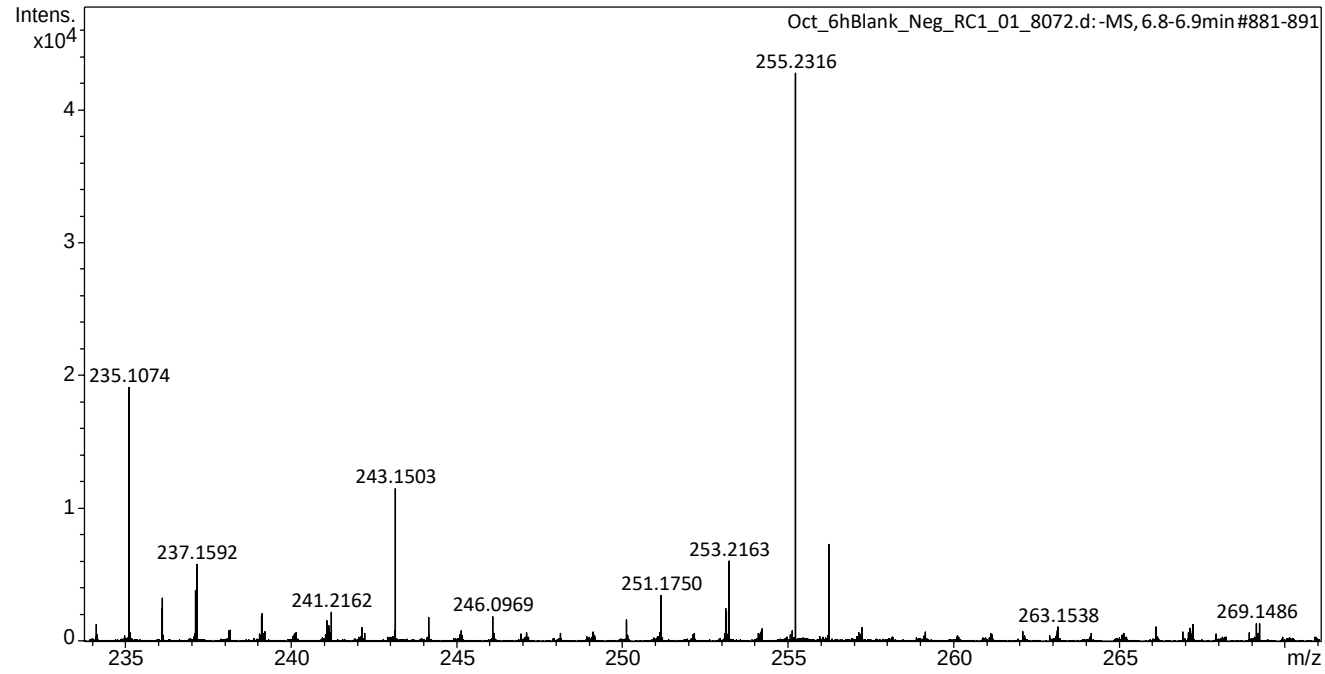
Sample Name Oct_6hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Oct_6hBlank_Neg_RC1_01_8072.d

Display Report

Analysis Info

Acquisition Date 16/3/2017 20:06:13

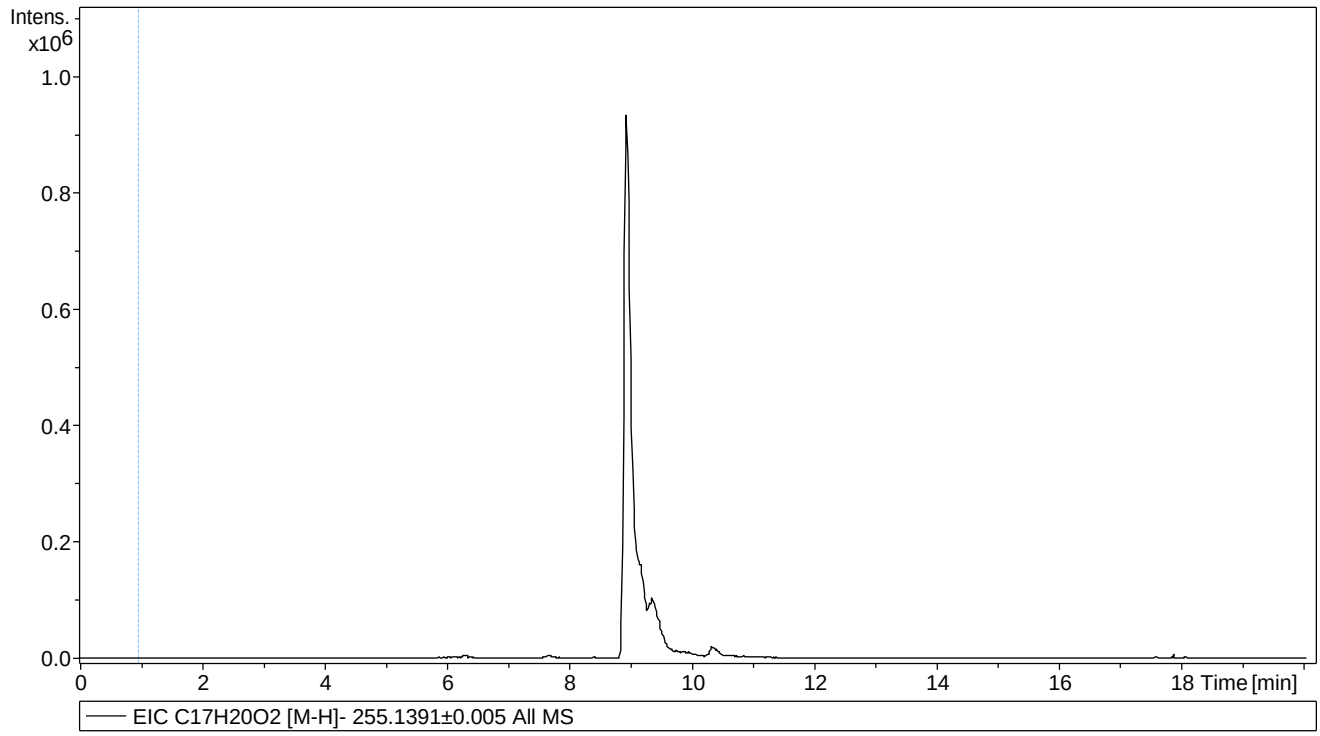
Operator BDAL@DE

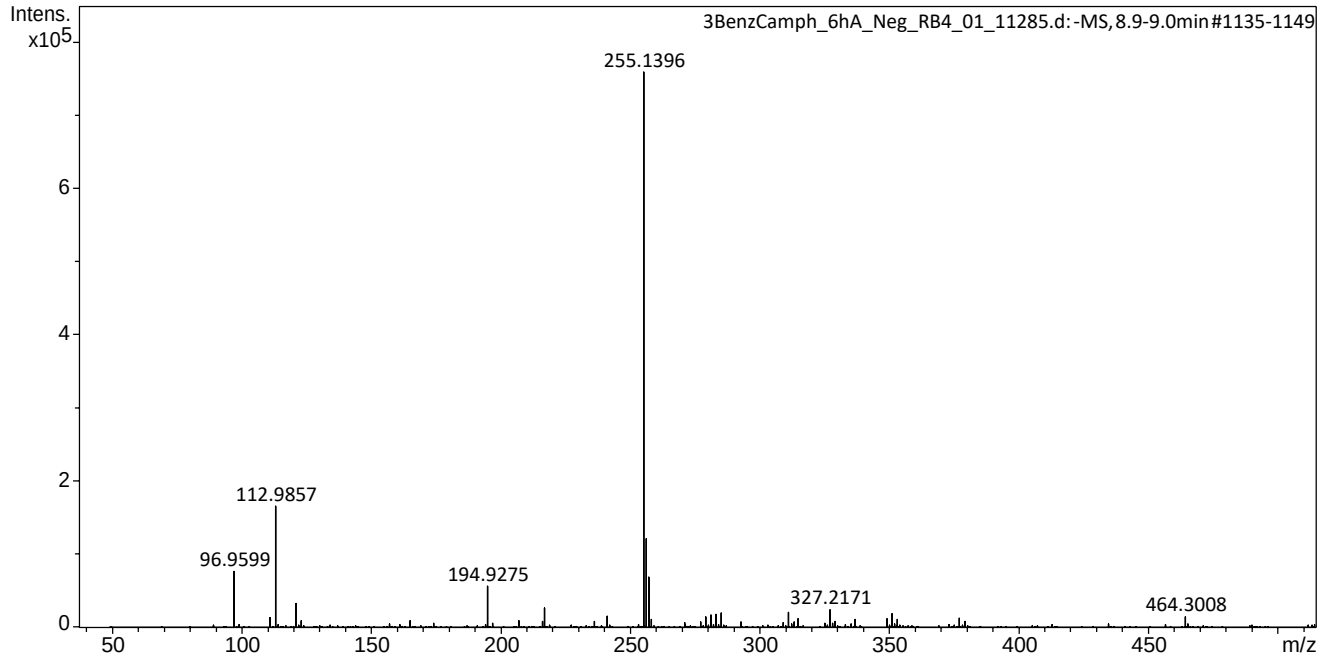
Sample Name 3BenzCamph_6hA_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 16/03/2017 20:27:28

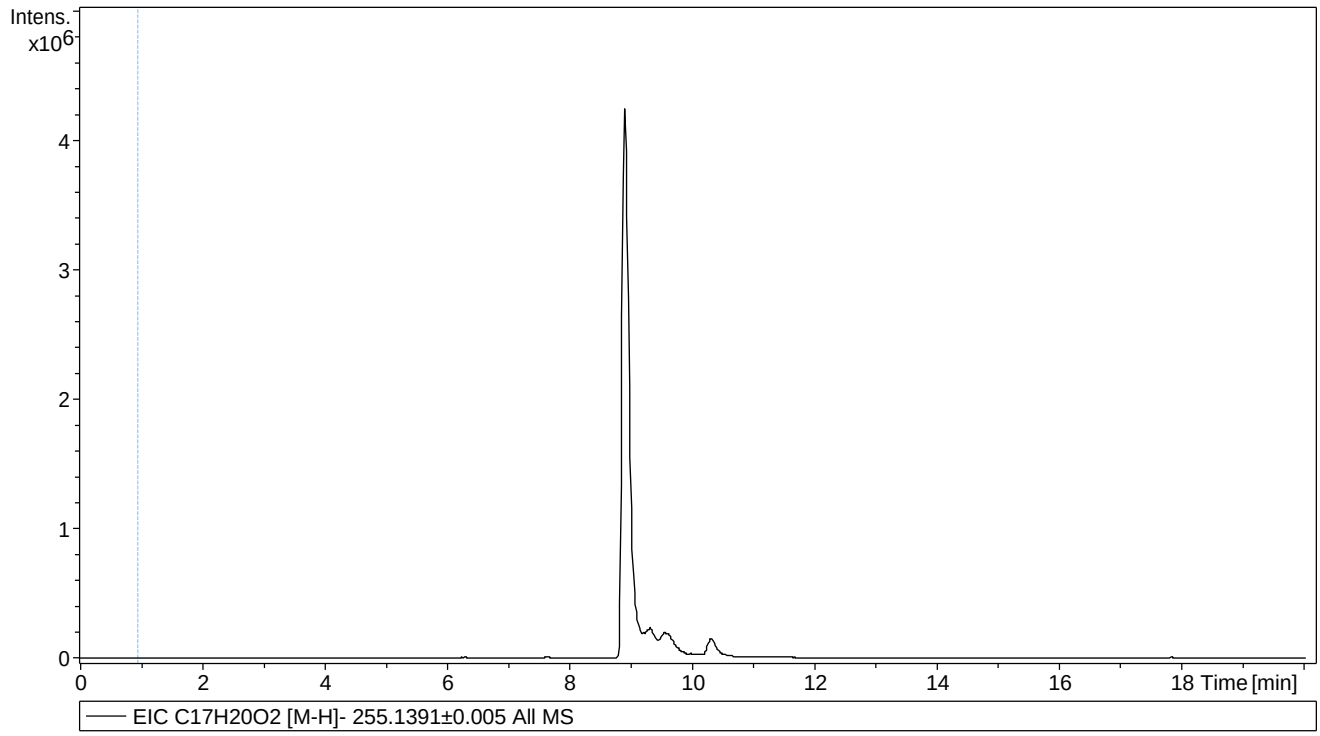
Operator BDAL@DE

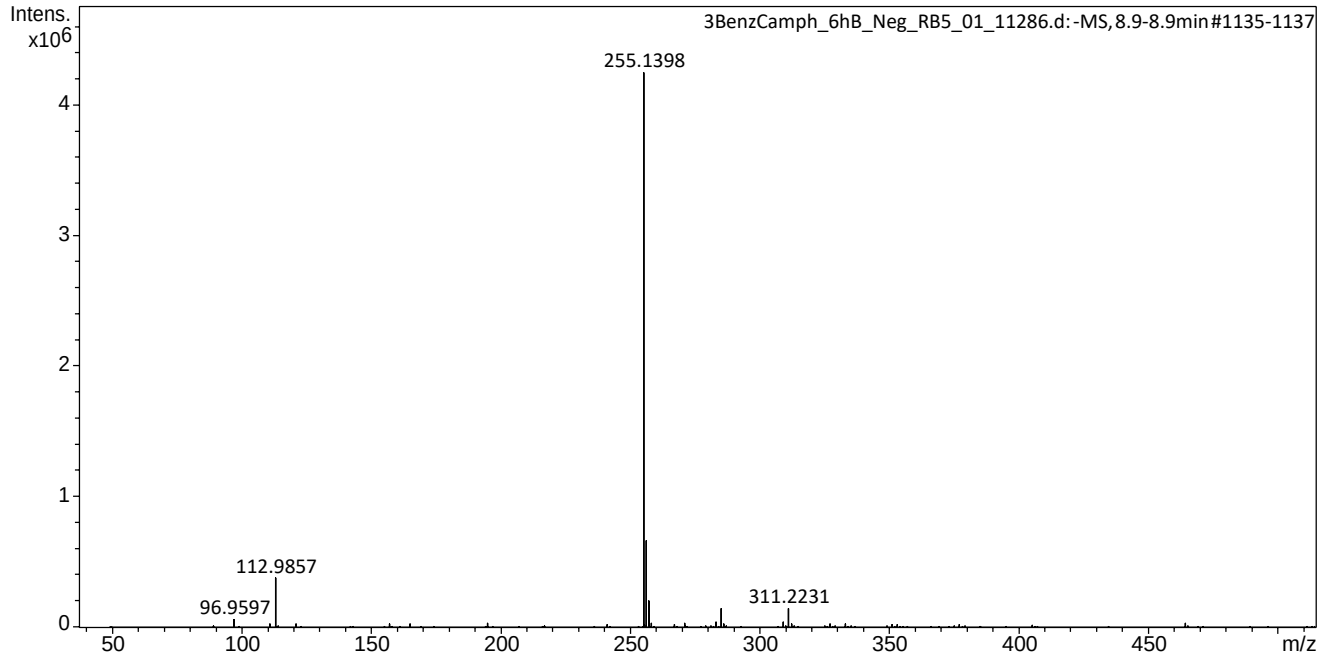
Sample Name 3BenzCamph_6hB_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 07/08/2017 18:58:40

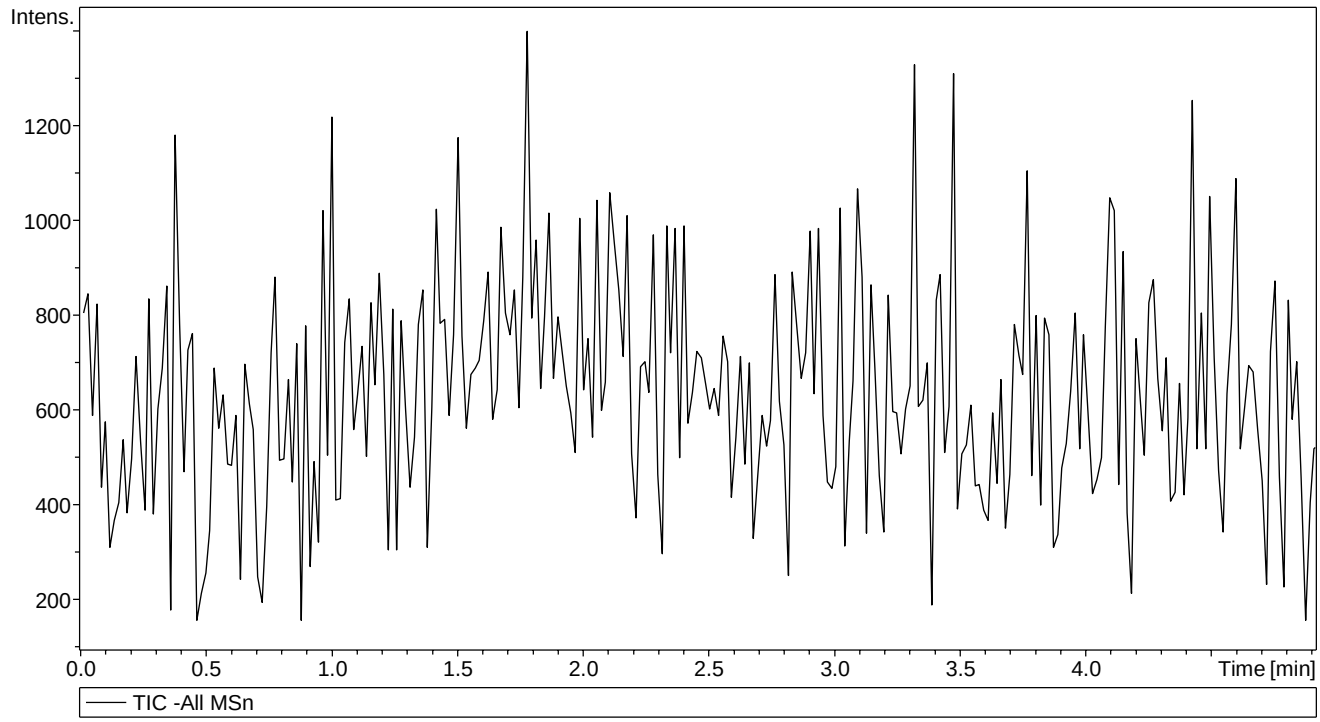
Operator BDAL@DE

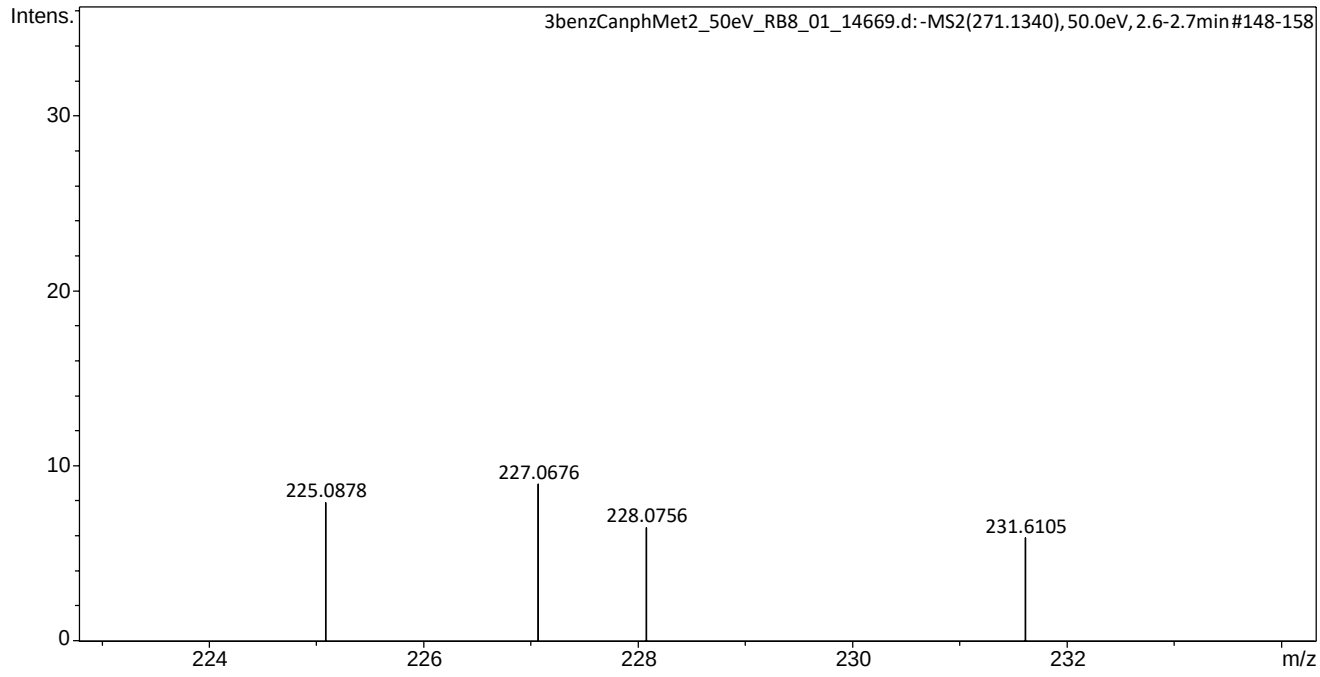
Sample Name 3benzCanphMet2_50eV

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	75 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





3benzCanphMet2_50eV_RB8_01_14669.d

Display Report

Analysis Info

Acquisition Date 07/08/2017 18:58:40

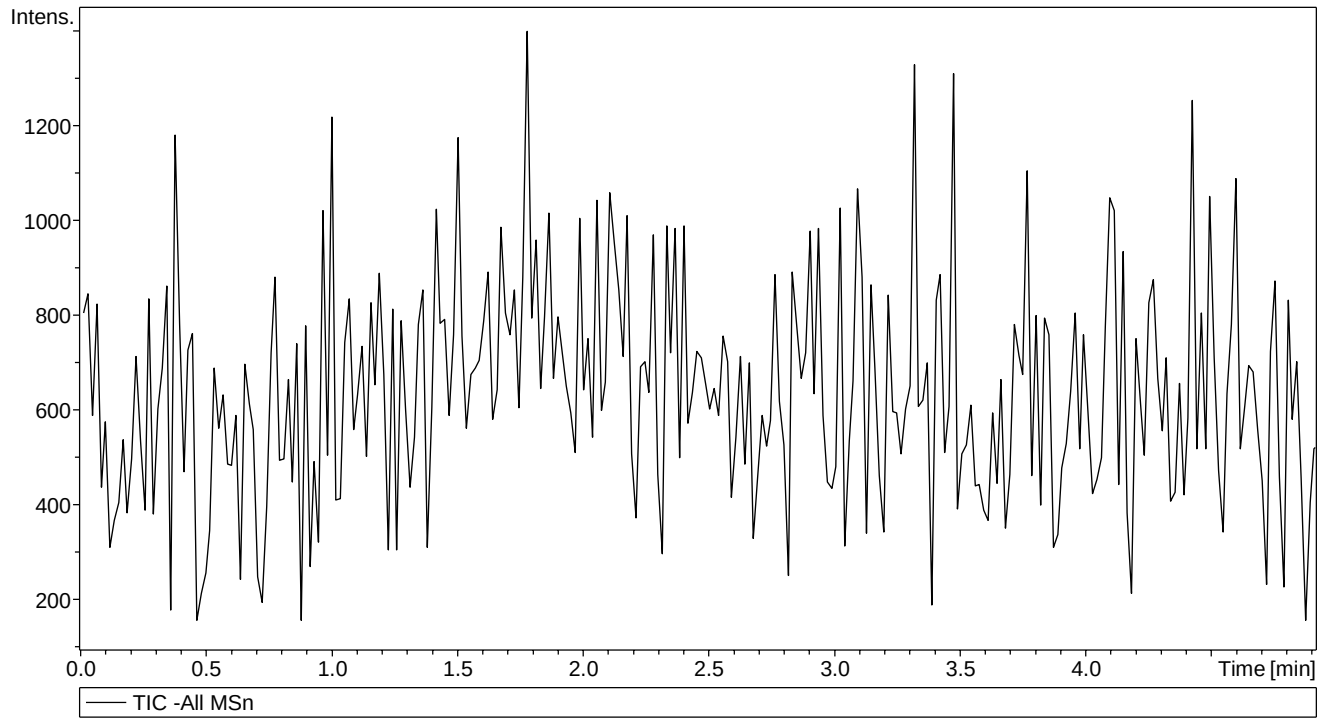
Operator BDAL@DE

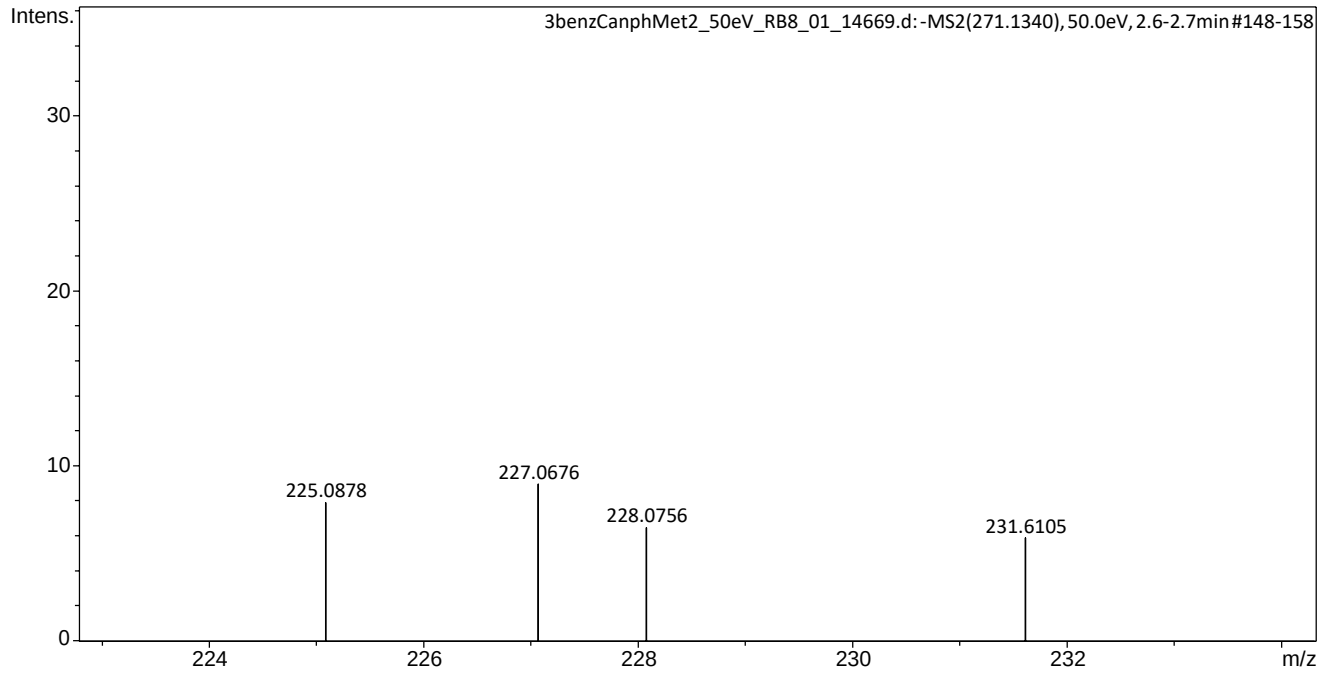
Sample Name 3benzCanphMet2_50eV

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	75 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	4000 nA	Set APCI Heater	200 °C





3benzCanphMet2_50eV_RB8_01_14669.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 21:03:25

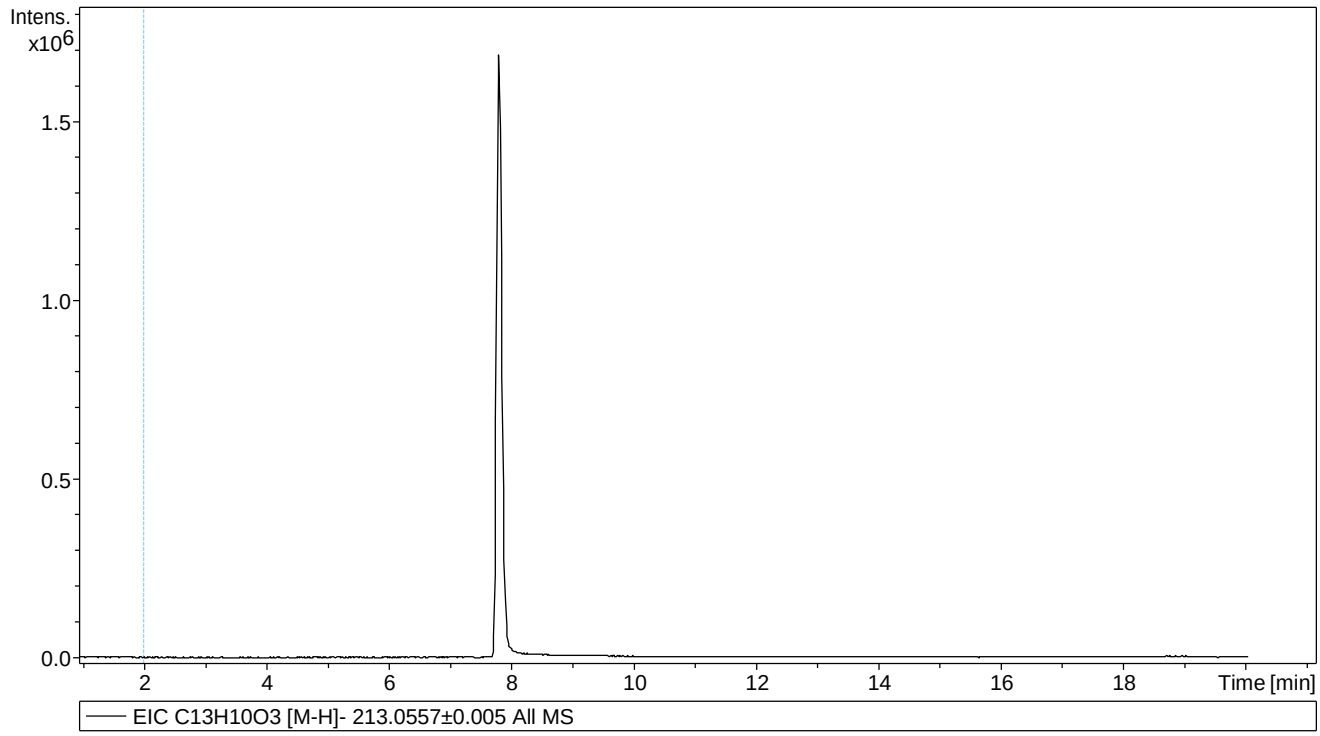
Operator BDAL@DE

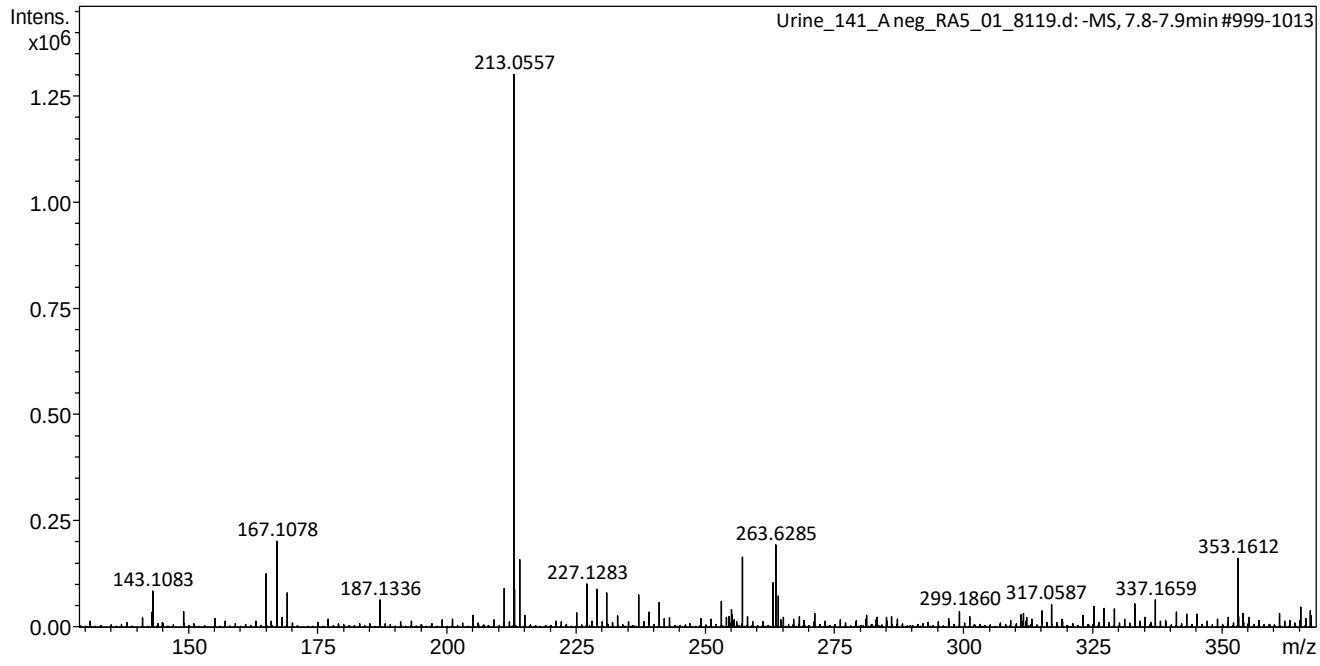
Sample Name Urine_141_A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_141_A neg_RA5_01_8119.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 21:45:56

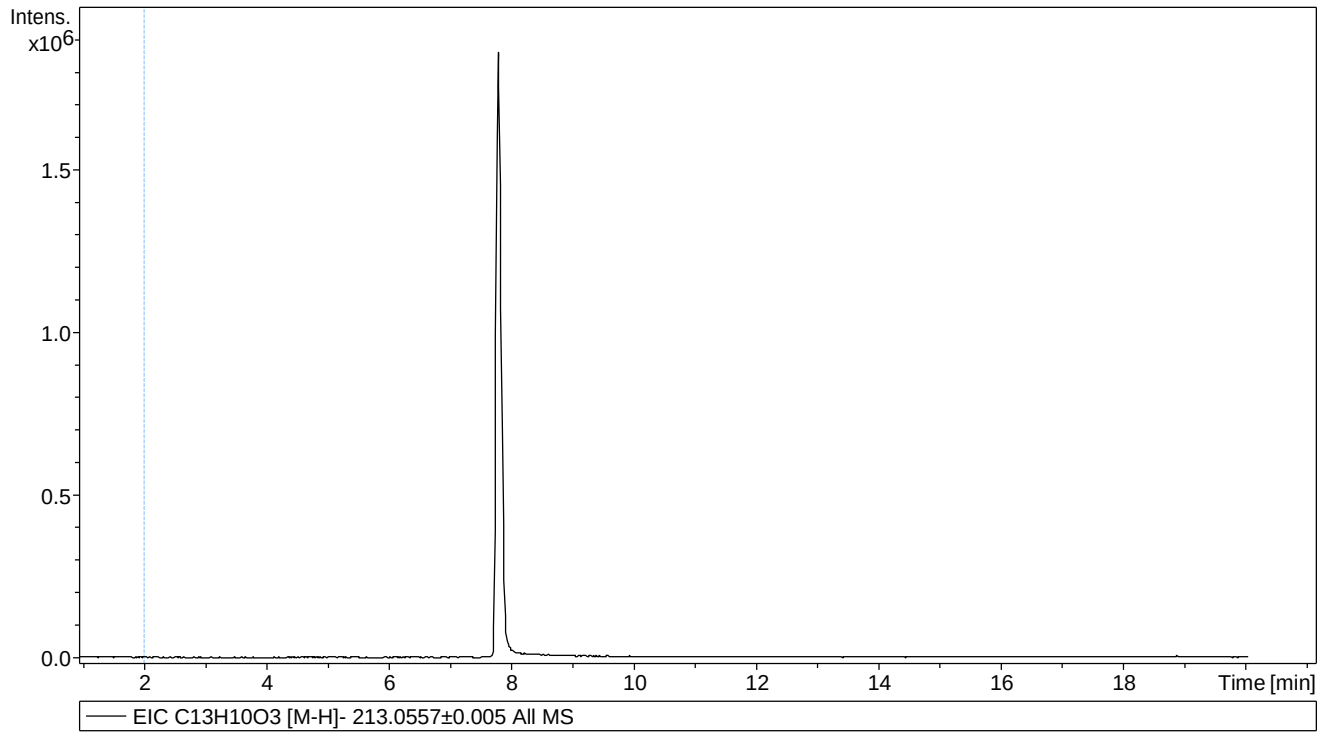
Operator BDAL@DE

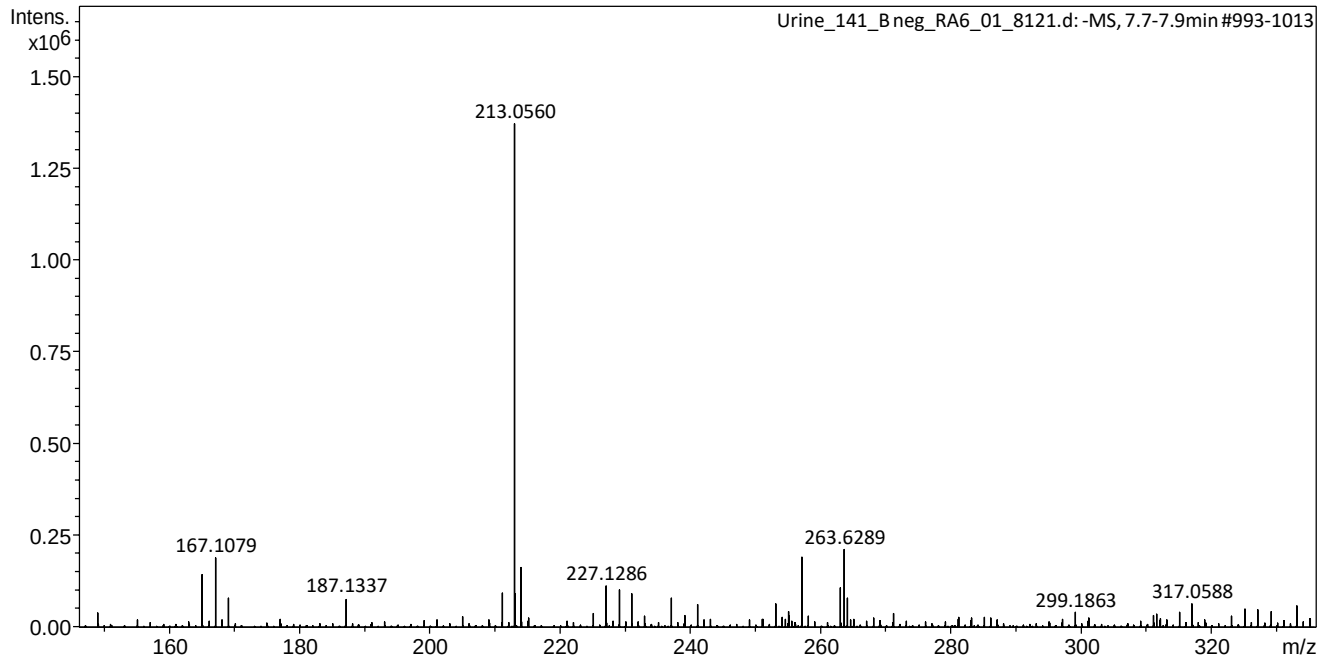
Sample Name Urine_141_B neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 14/03/2017 21:08:18

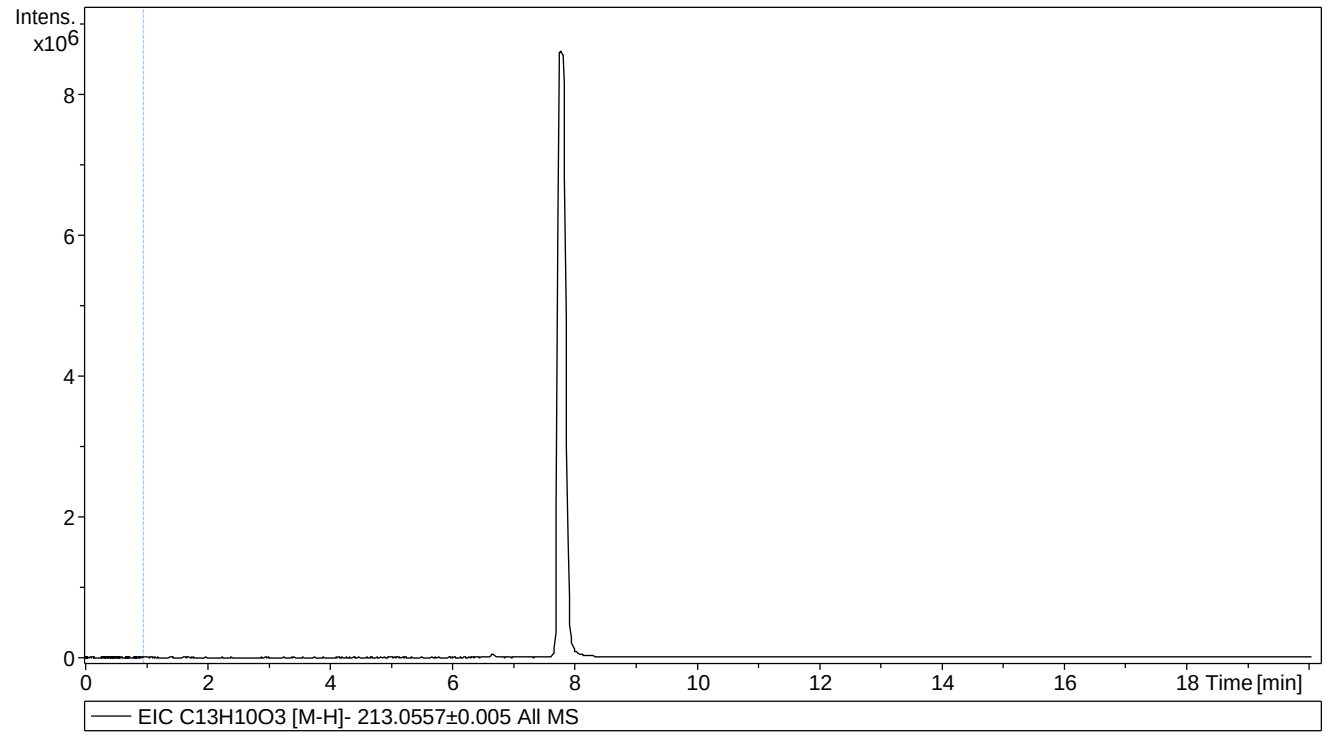
Operator BDAL@DE

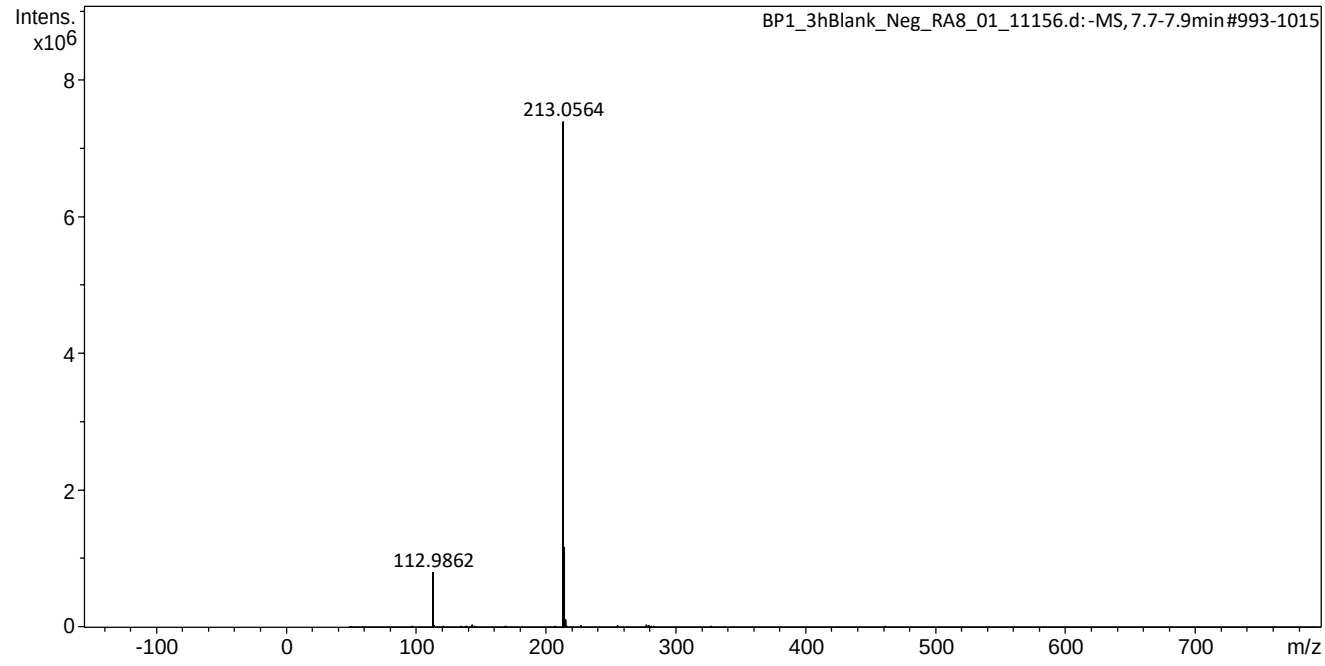
Sample Name BP1_3hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 24/11/2016 03:25:59

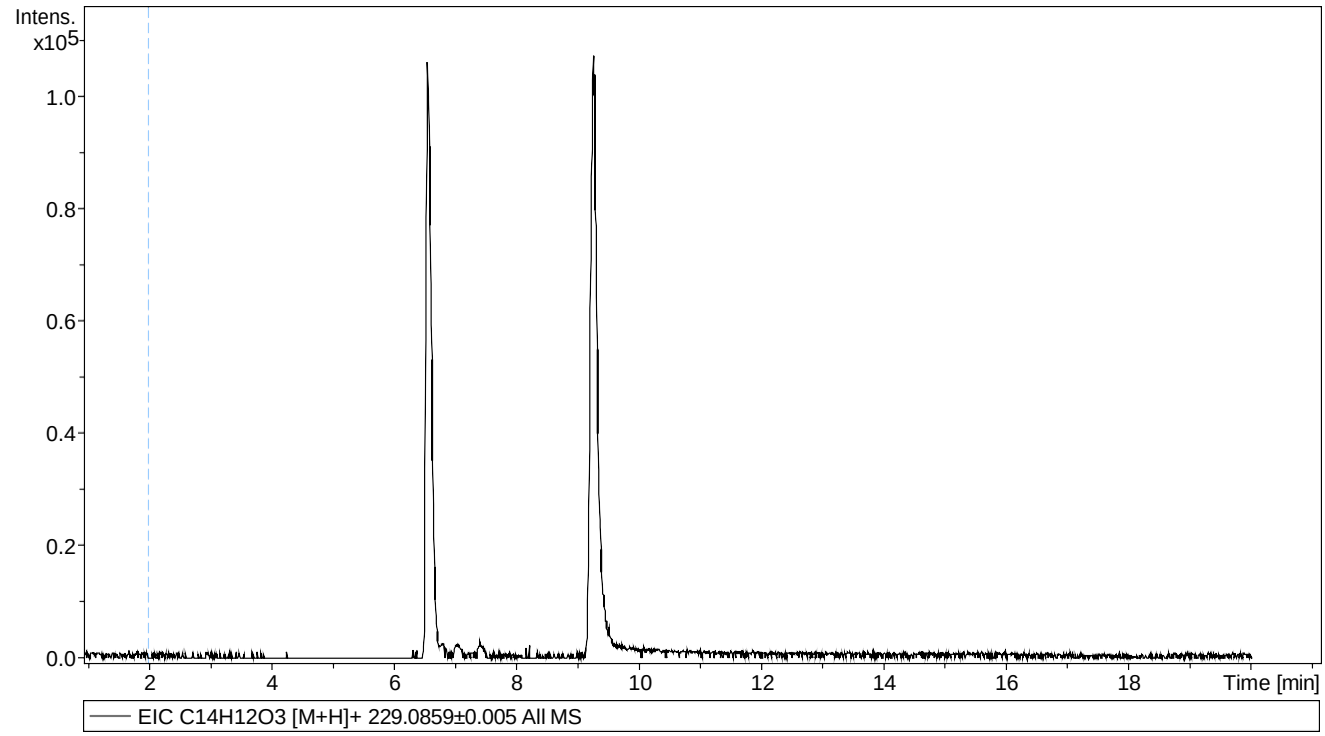
Operator BDAL@DE

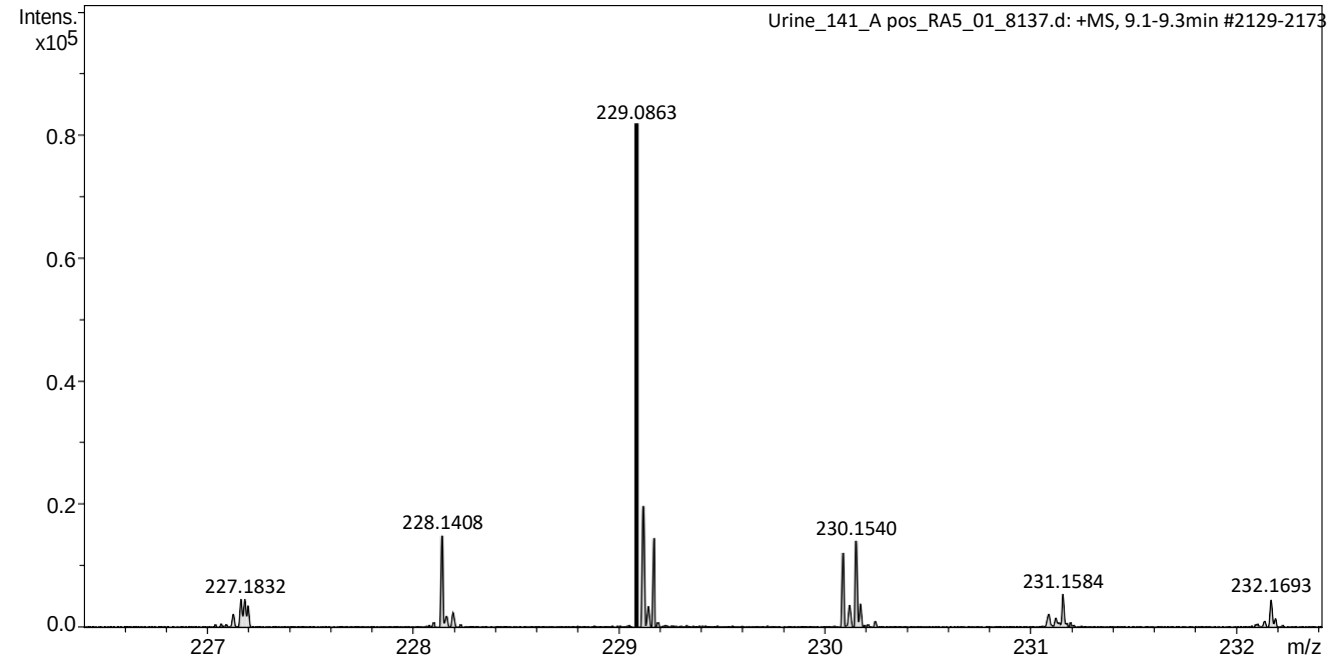
Sample Name Urine_141_A pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 24/11/2016 04:08:30

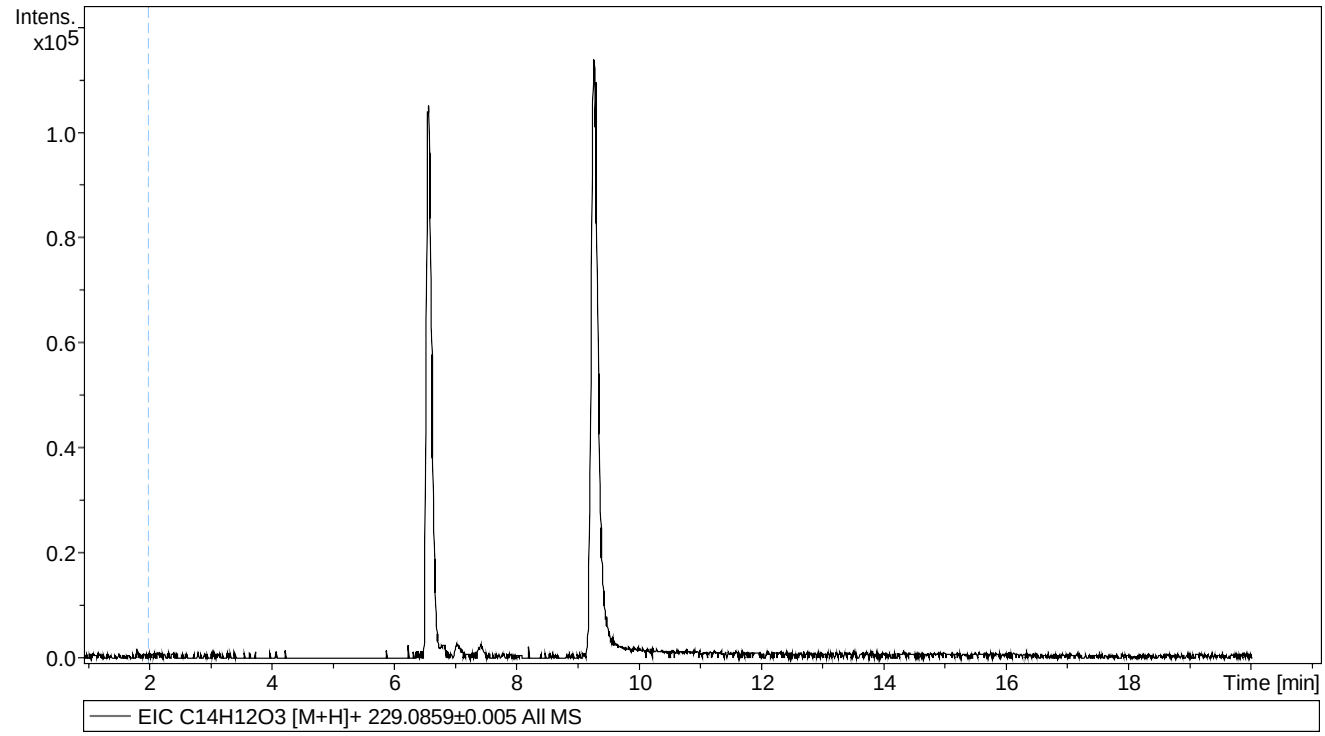
Operator BDAL@DE

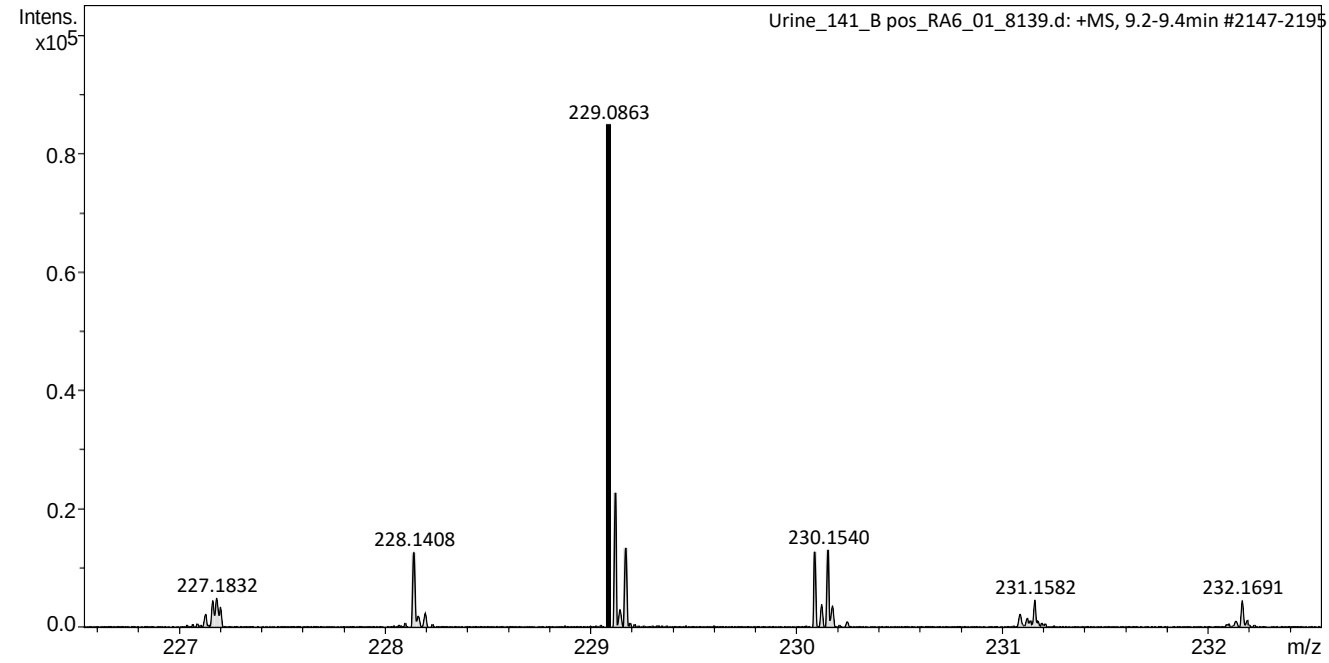
Sample Name Urine_141_B pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 24/11/2016 02:43:30

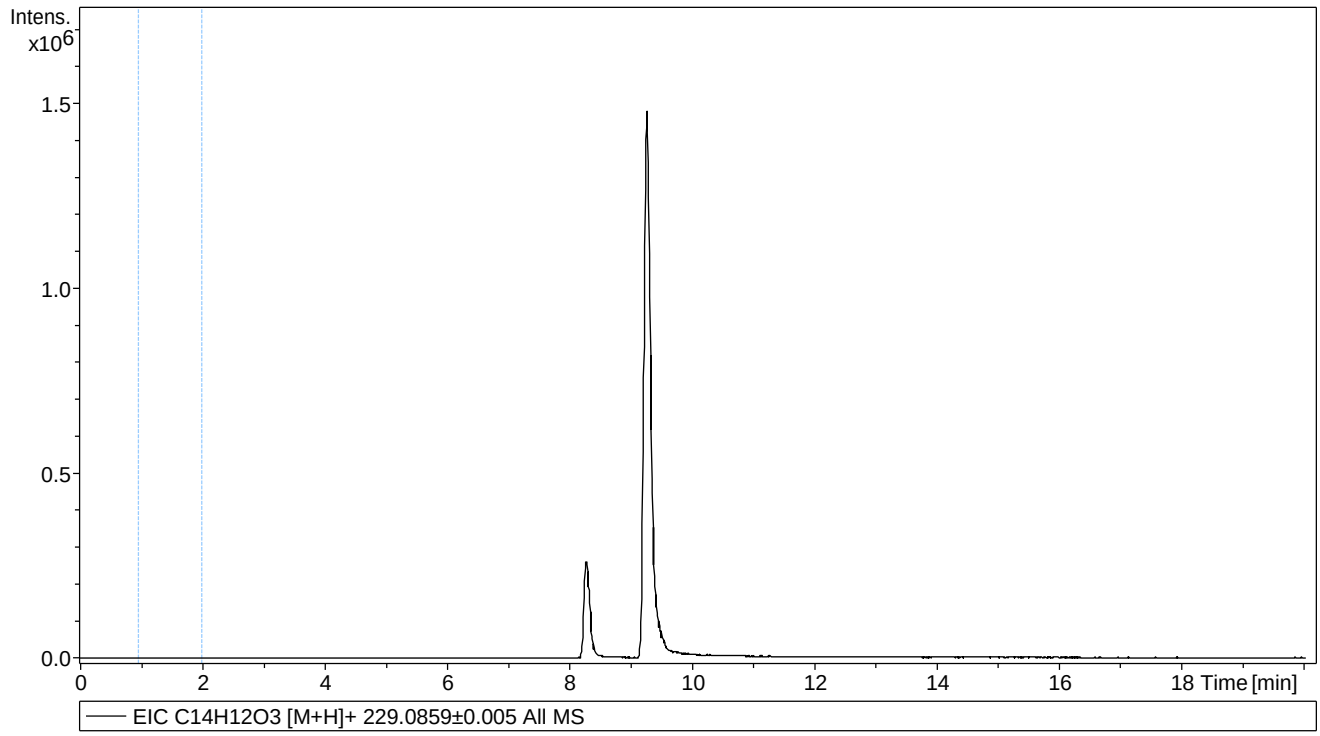
Operator BDAL@DE

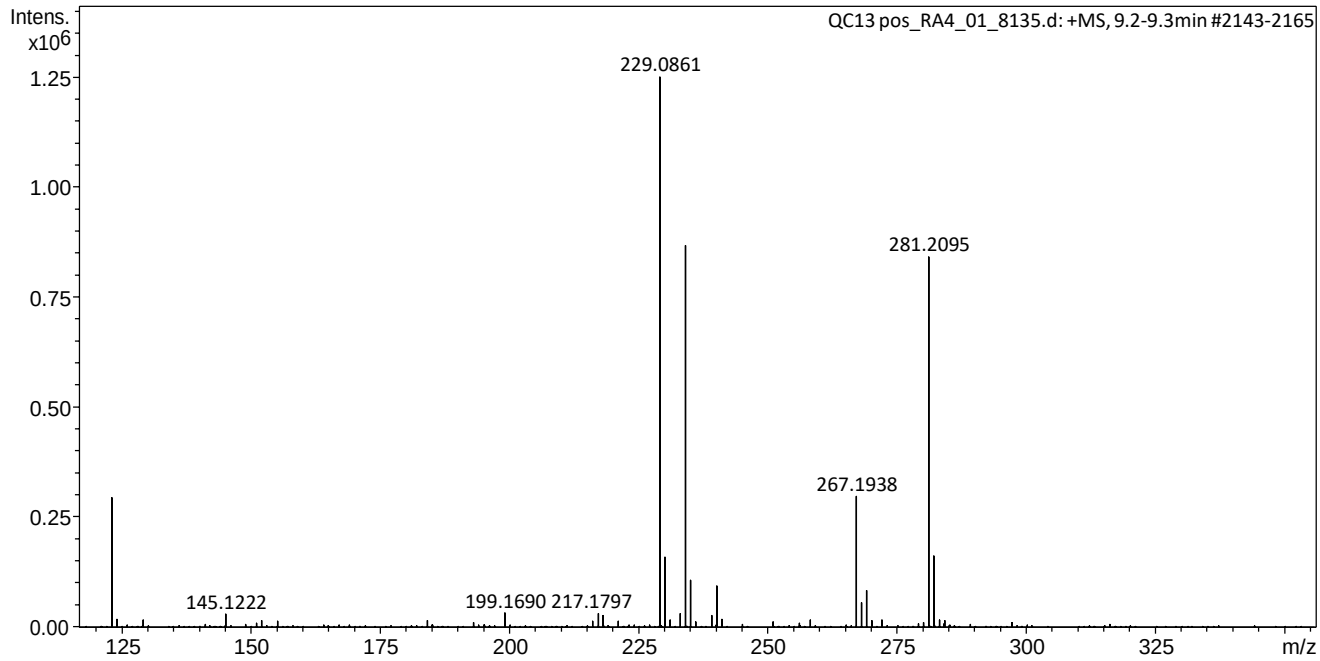
Sample Name QC13 pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 22:28:28

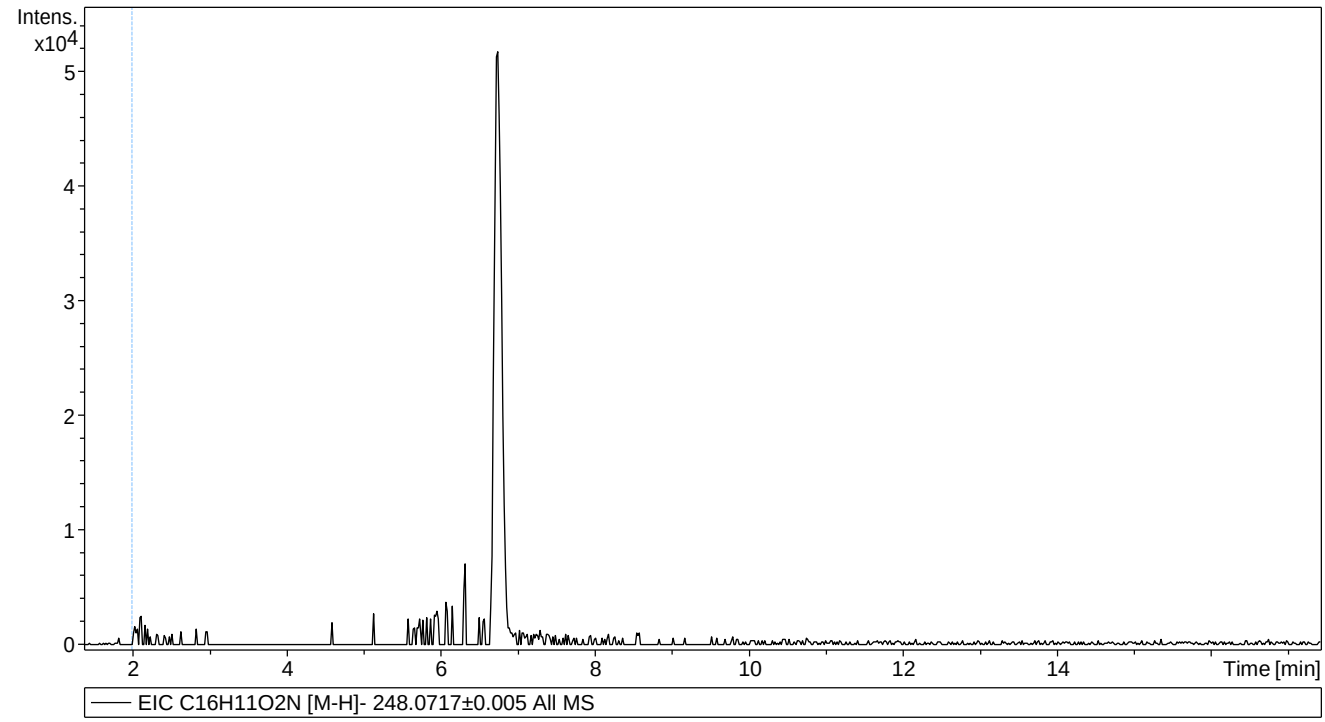
Operator BDAL@DE

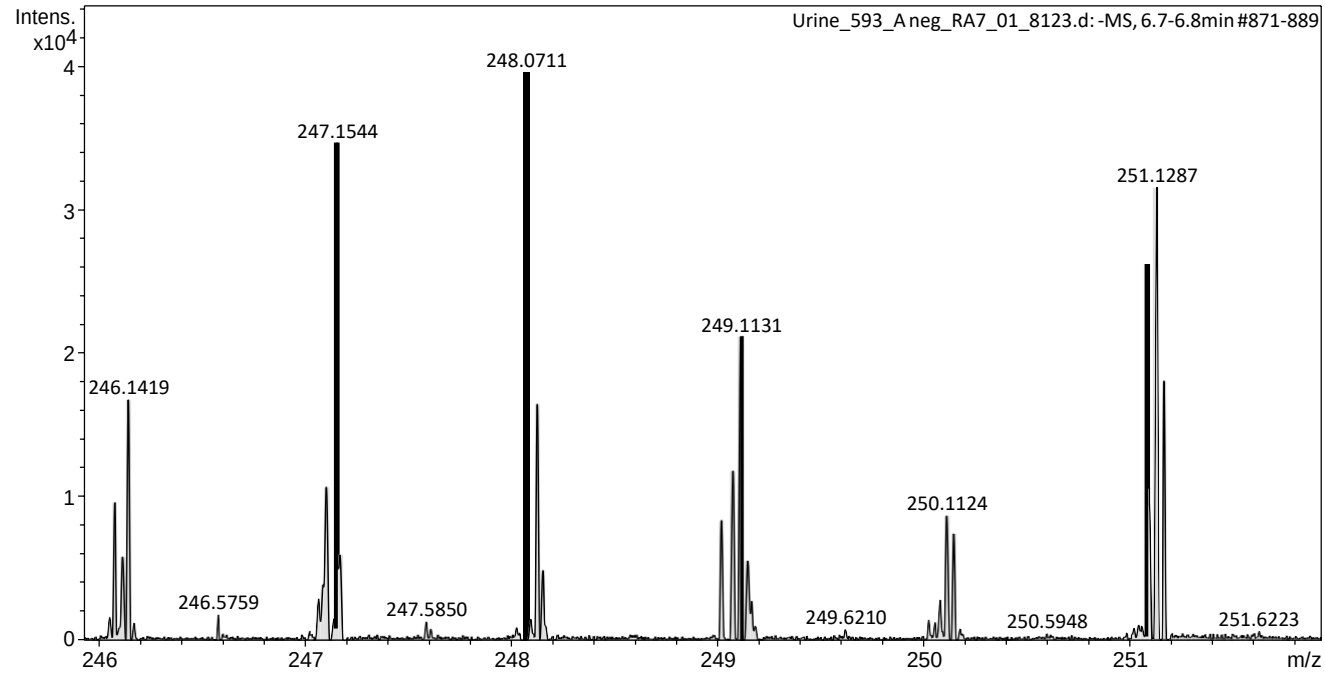
Sample Name Urine_593_A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_A neg_RA7_01_8123.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 22:28:28

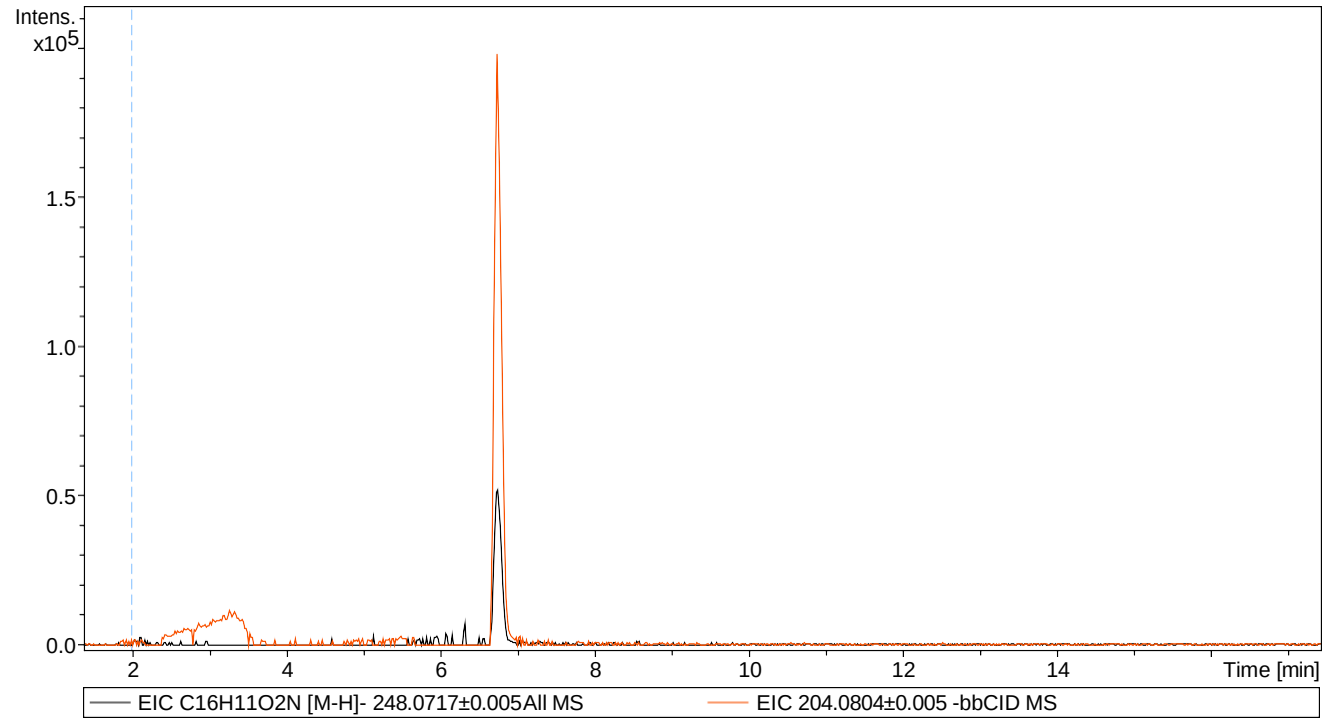
Operator BDAL@DE

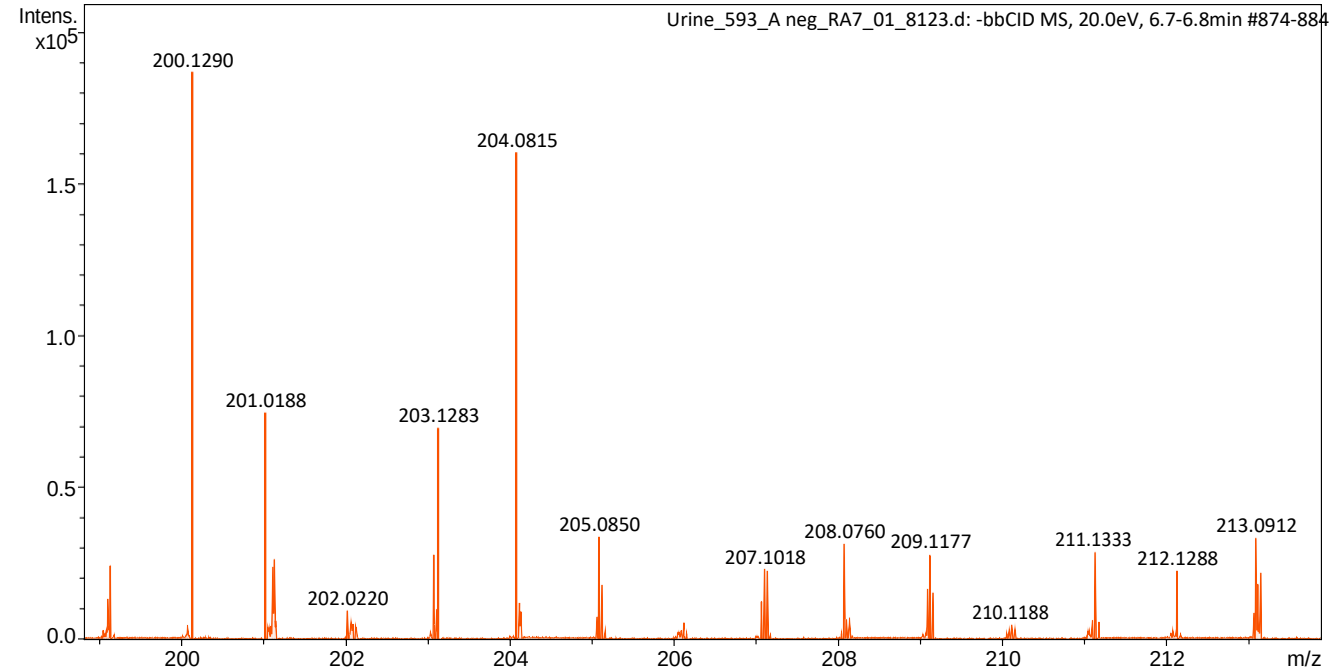
Sample Name Urine_593_A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 23:10:56

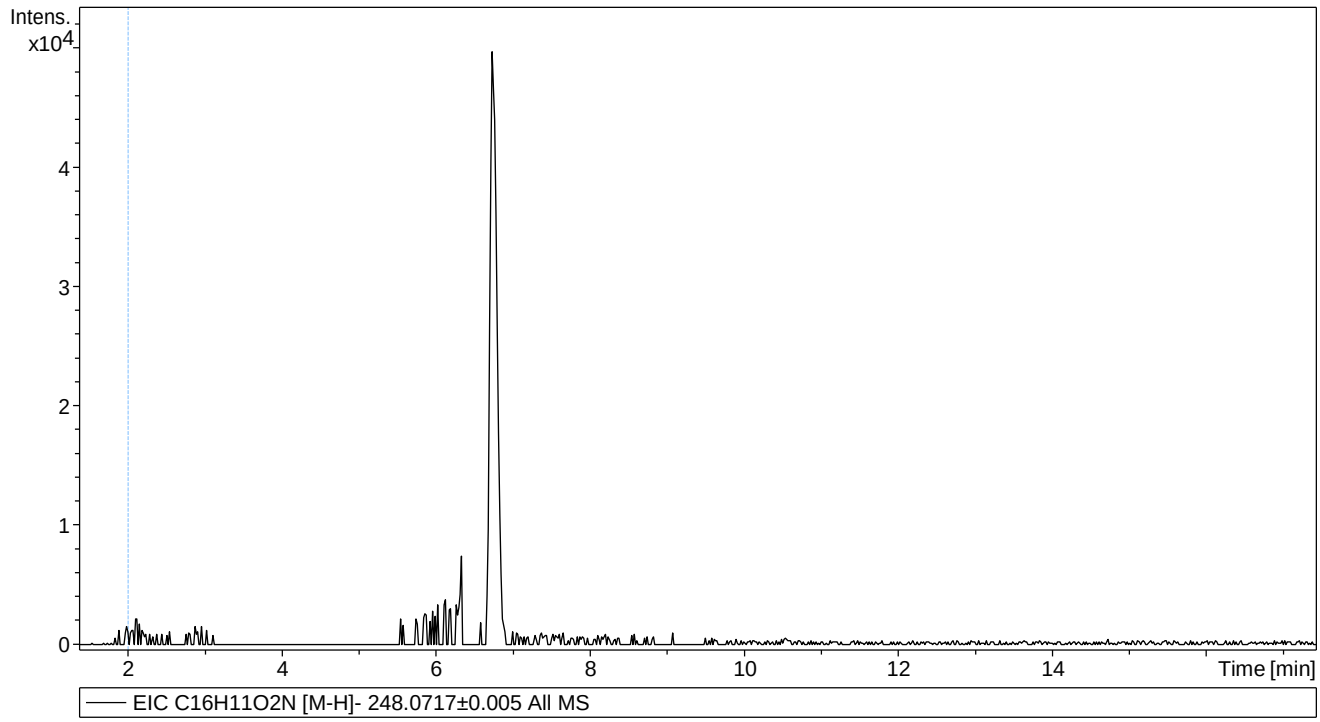
Operator BDAL@DE

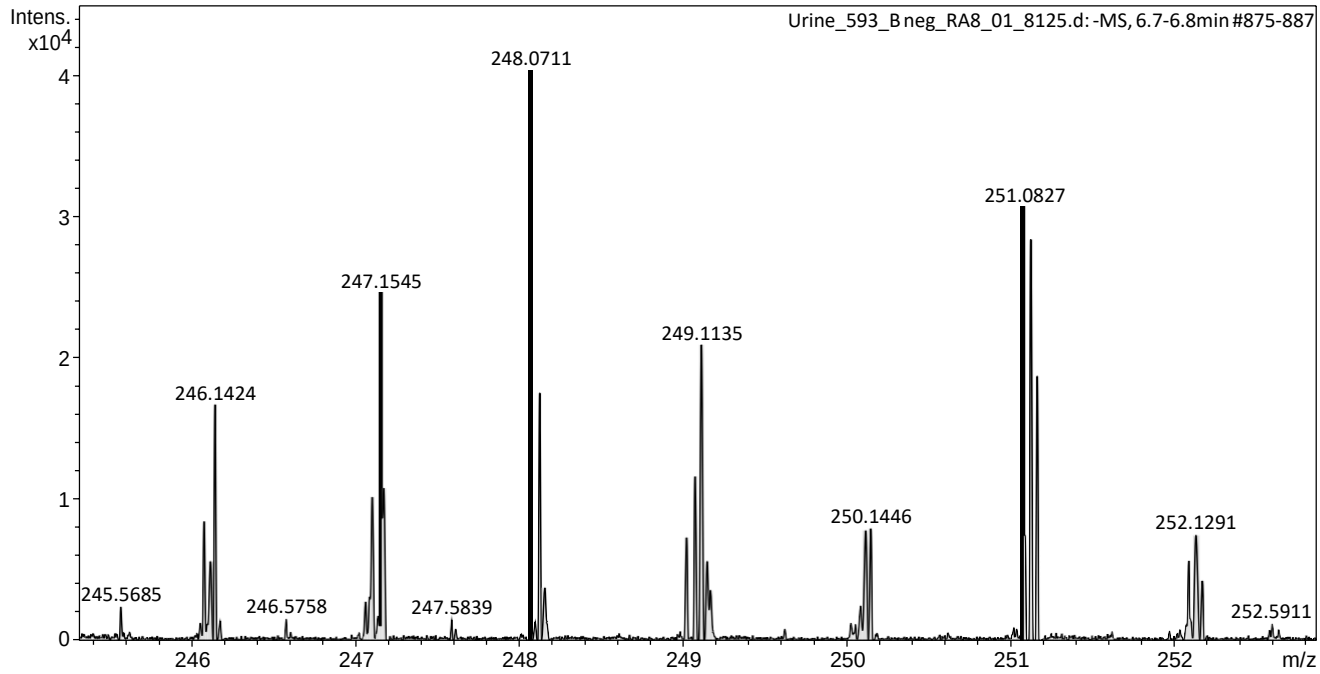
Sample Name Urine_593_B neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_B neg_RA8_01_8125.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 23:10:56

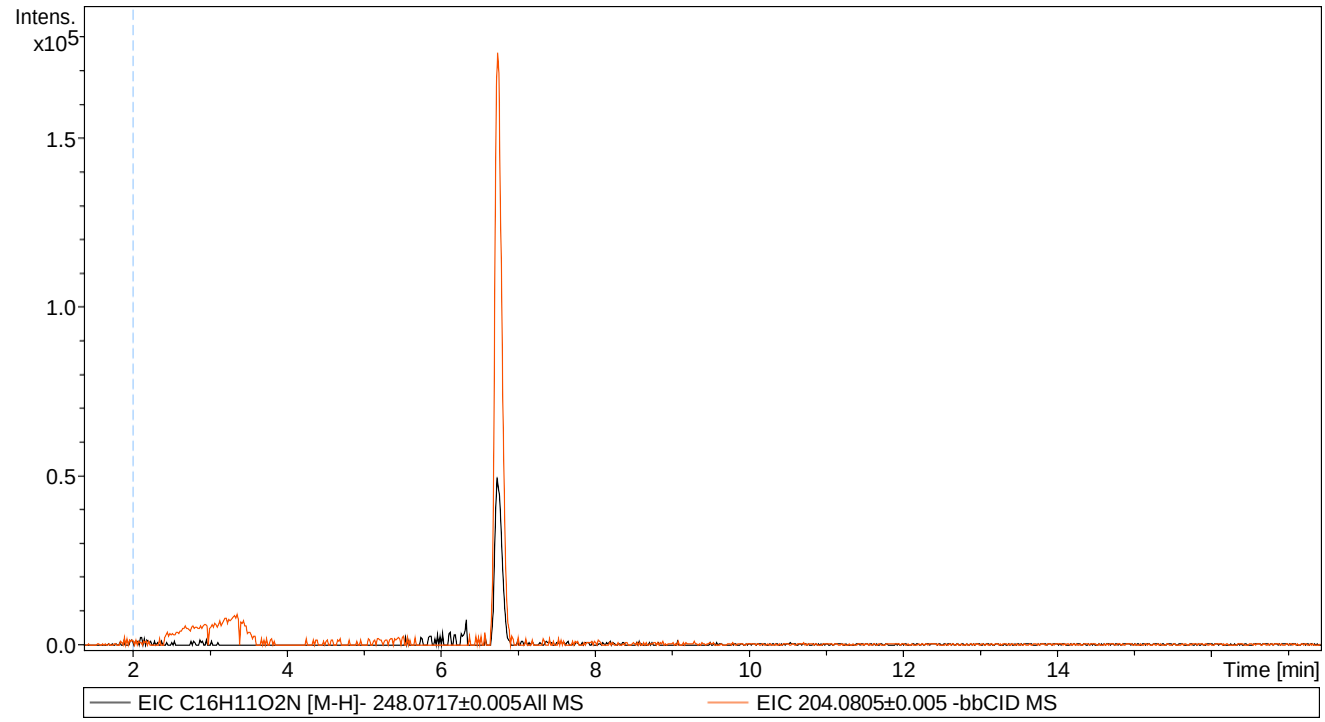
Operator BDAL@DE

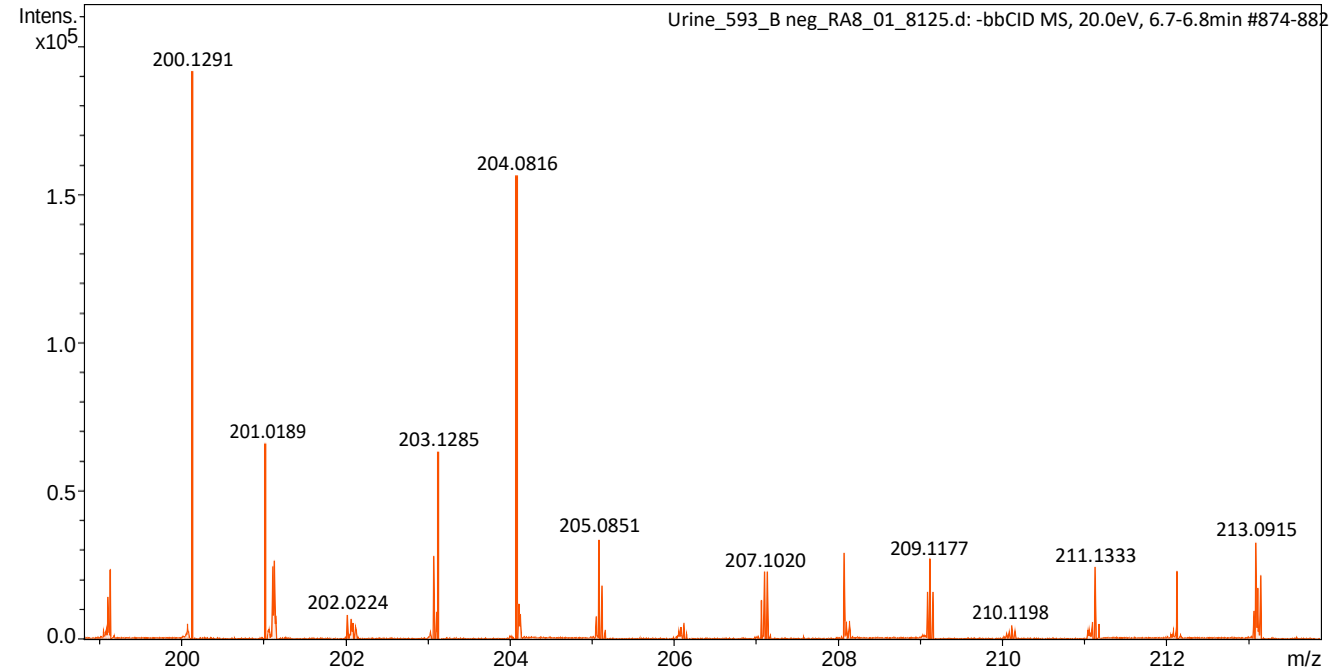
Sample Name Urine_593_B neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/11/2016 22:28:28

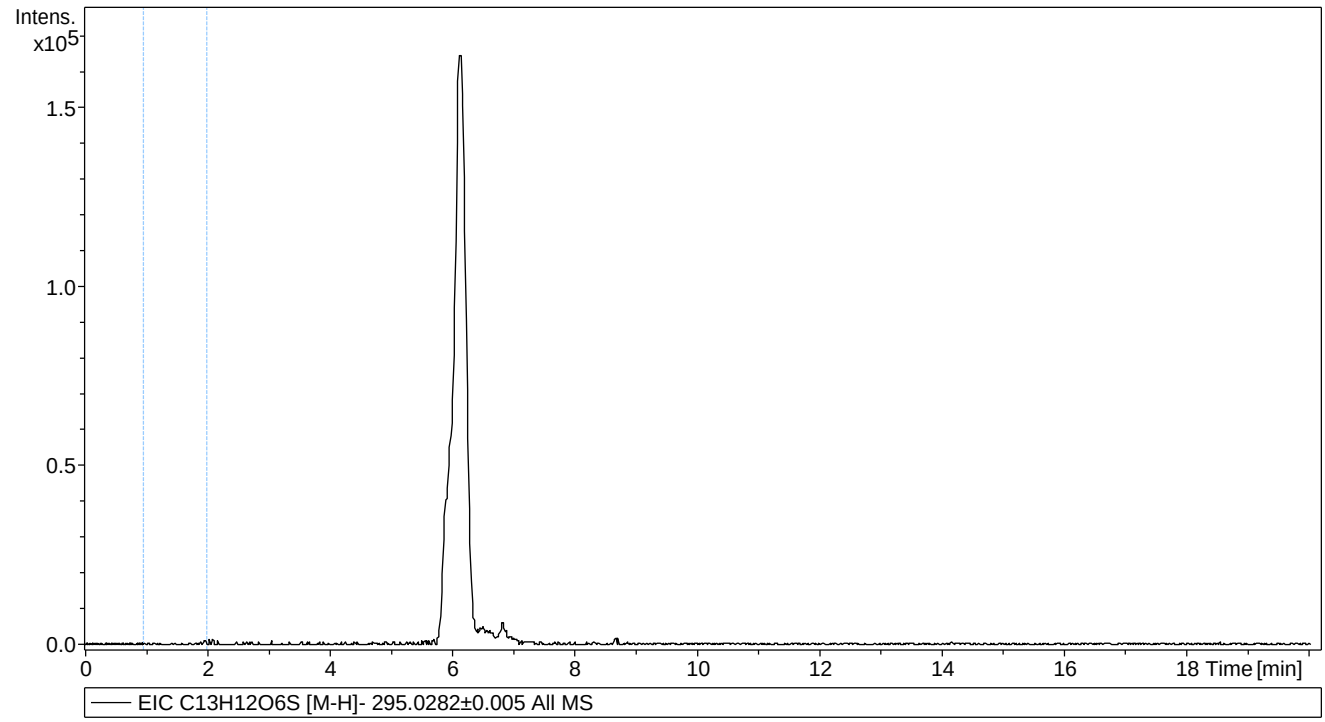
Operator BDAL@DE

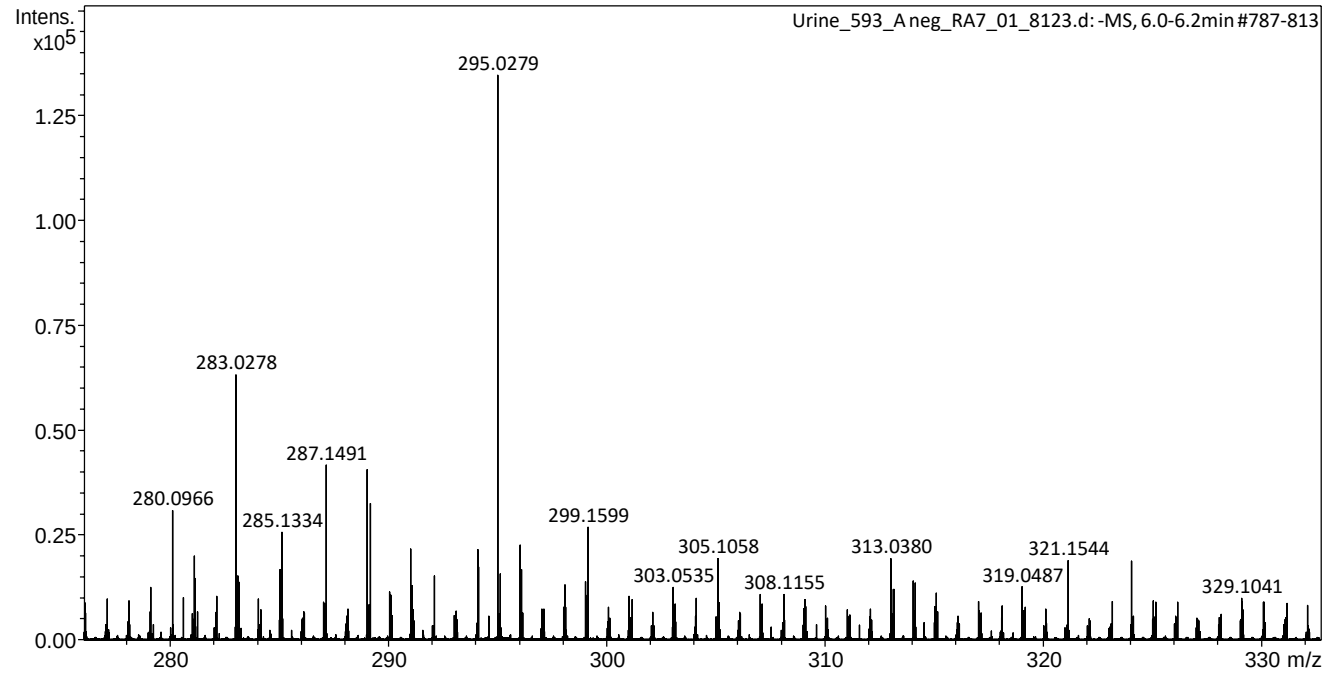
Sample Name Urine_593_A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_A neg_RA7_01_8123.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 22:28:28

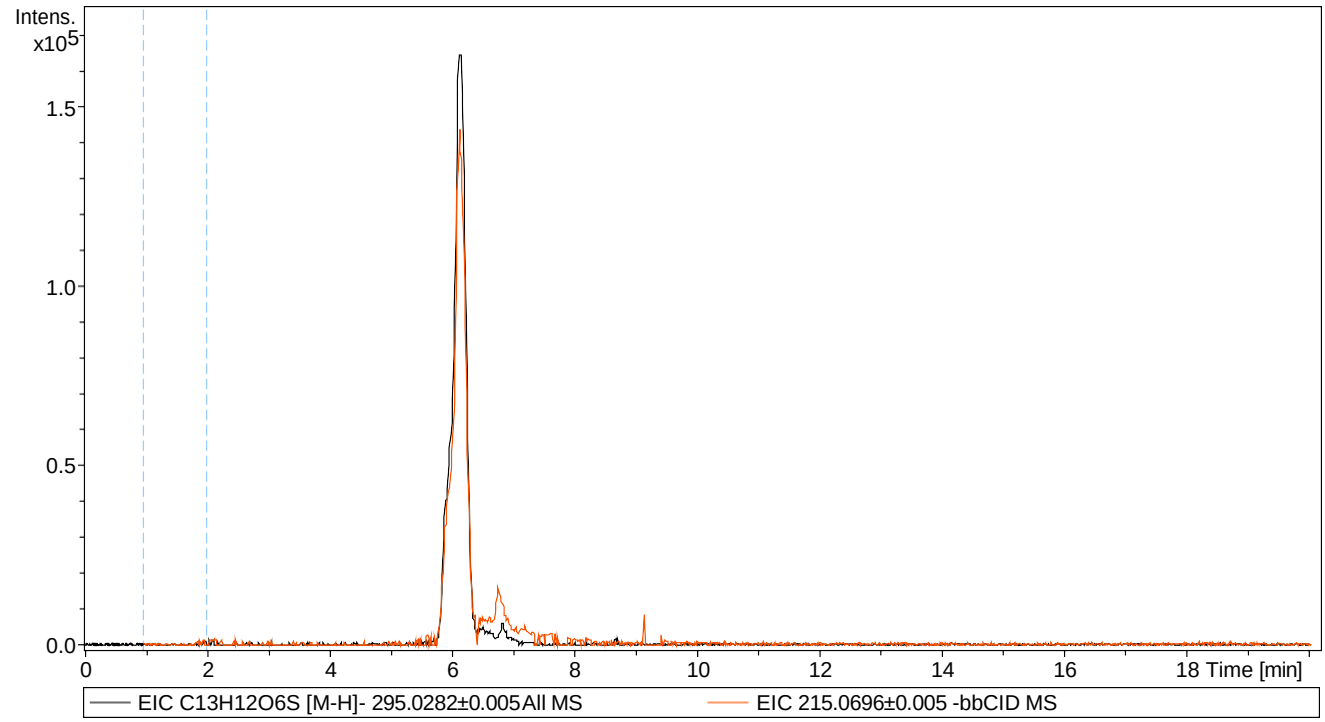
Operator BDAL@DE

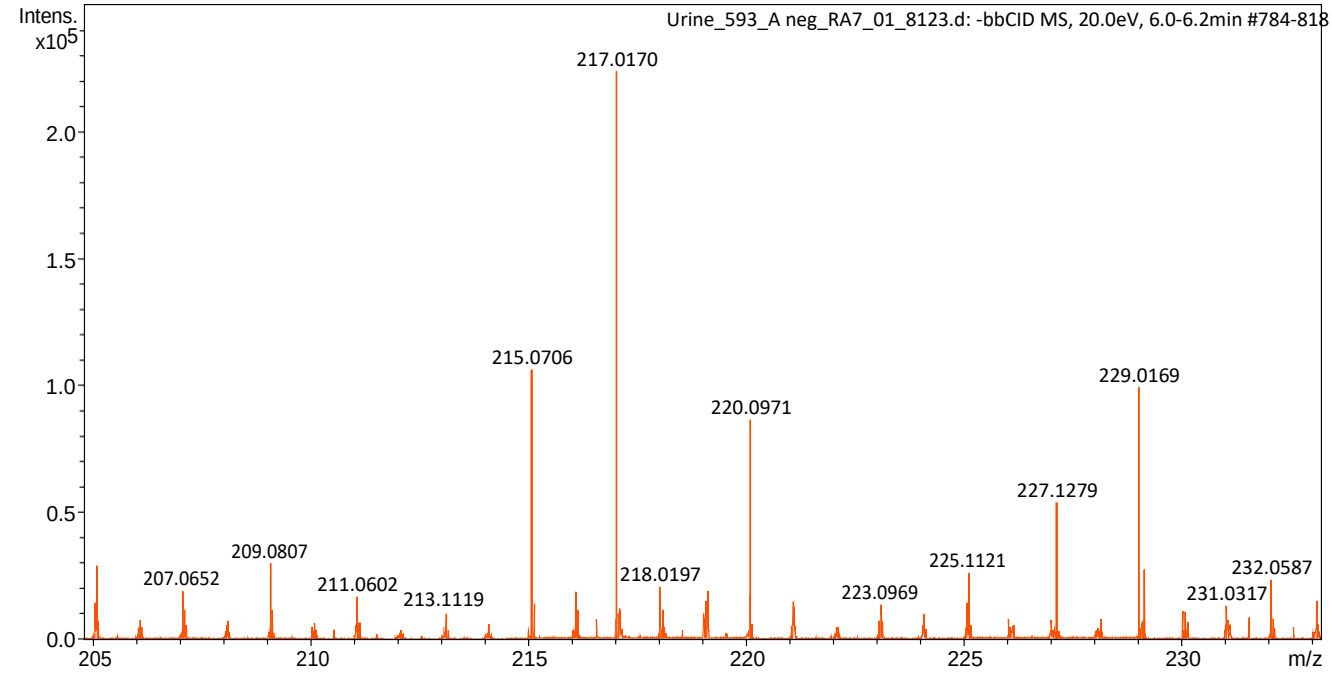
Sample Name Urine_593_A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_A neg_RA7_01_8123.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 23:10:56

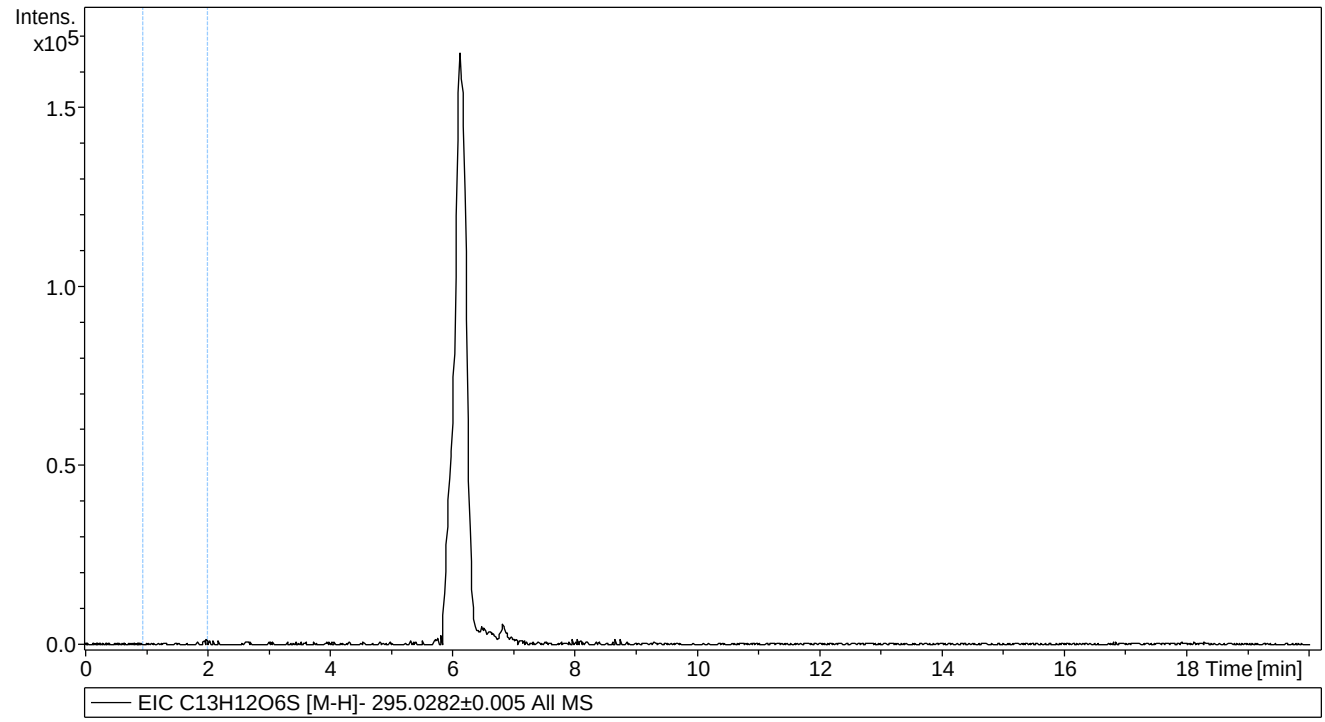
Operator BDAL@DE

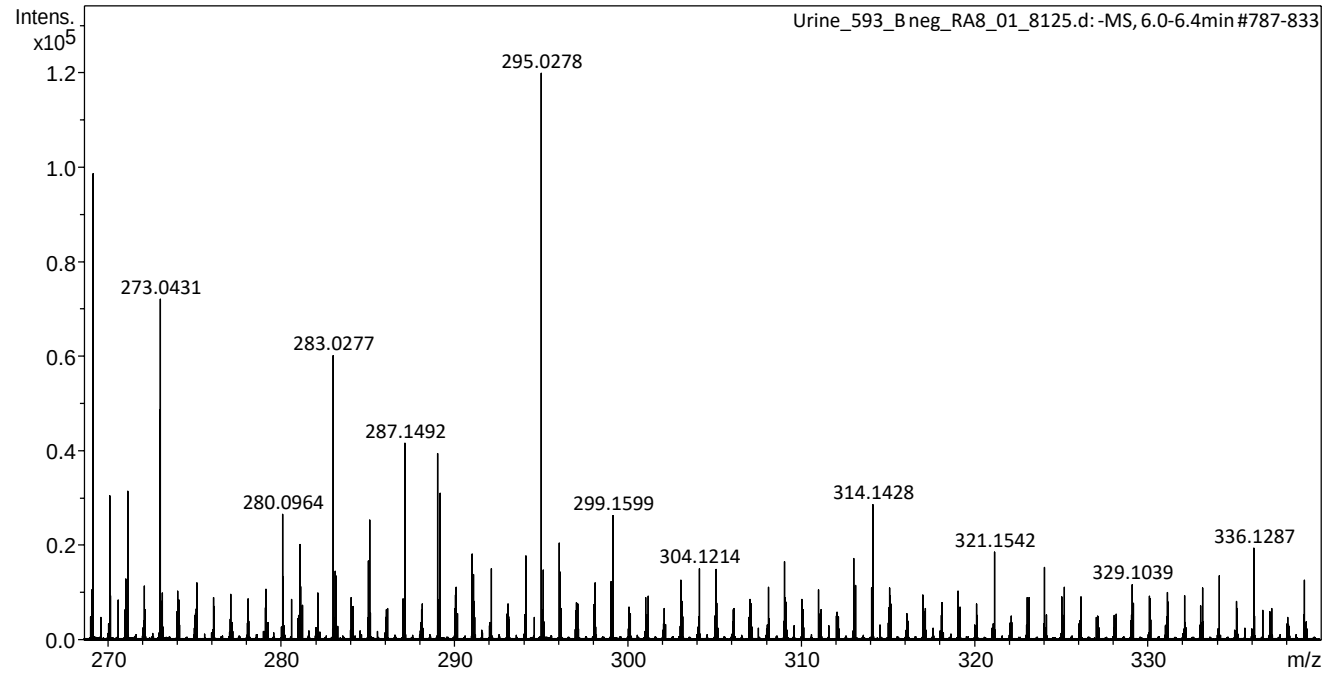
Sample Name Urine_593_B neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_B neg_RA8_01_8125.d

Display Report

Analysis Info

Acquisition Date 23/11/2016 23:10:56

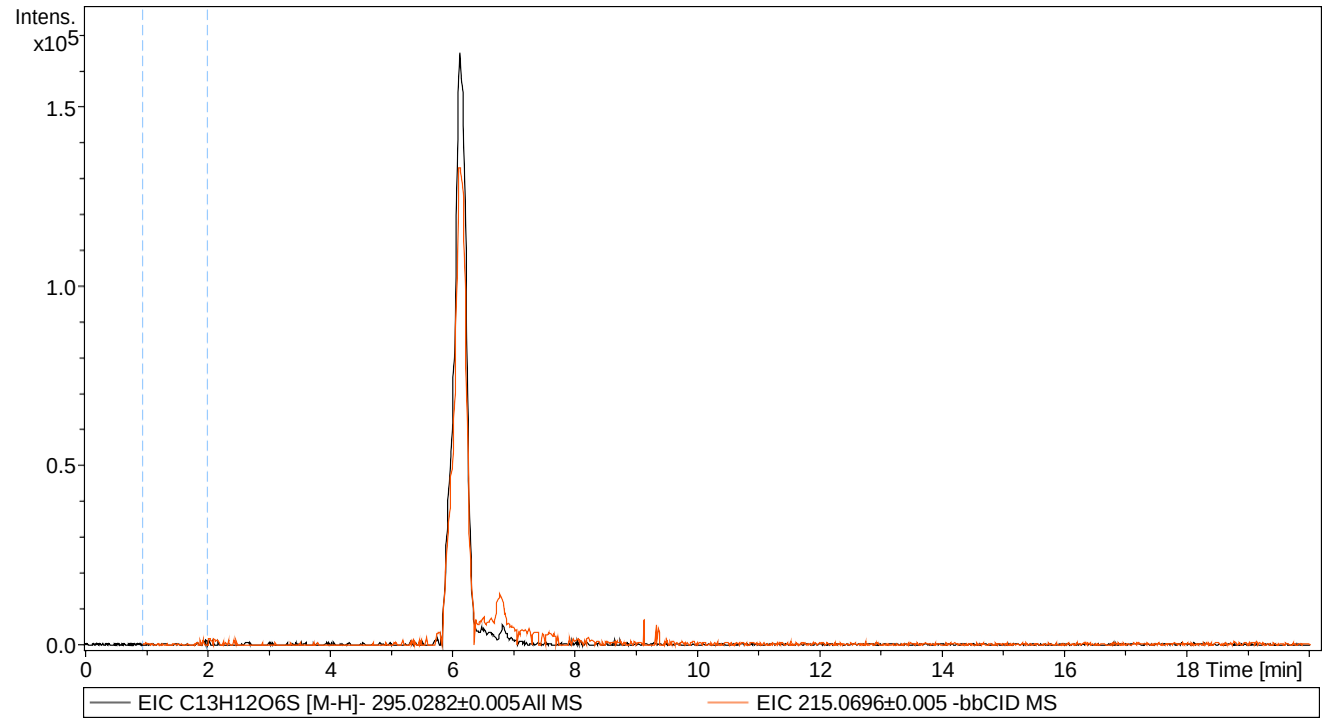
Operator BDAL@DE

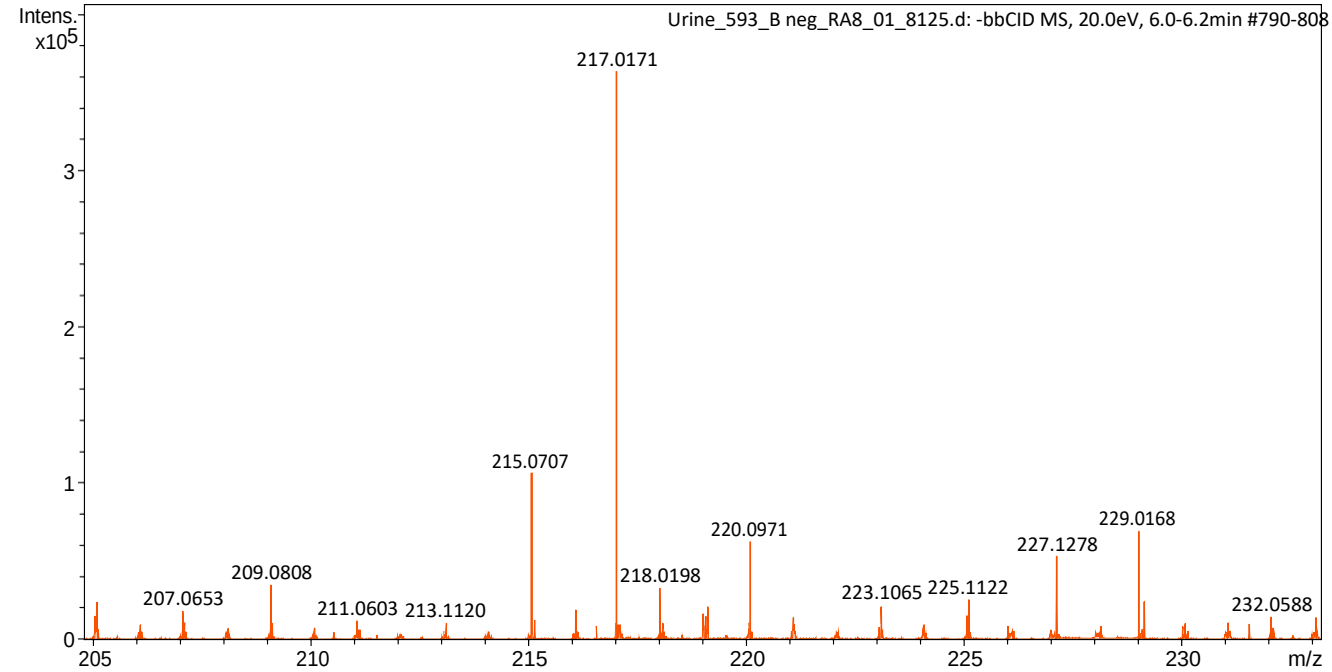
Sample Name Urine_593_B neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Urine_593_B neg_RA8_01_8125.d

Display Report

Analysis Info

Acquisition Date 22/10/201606 :41:01

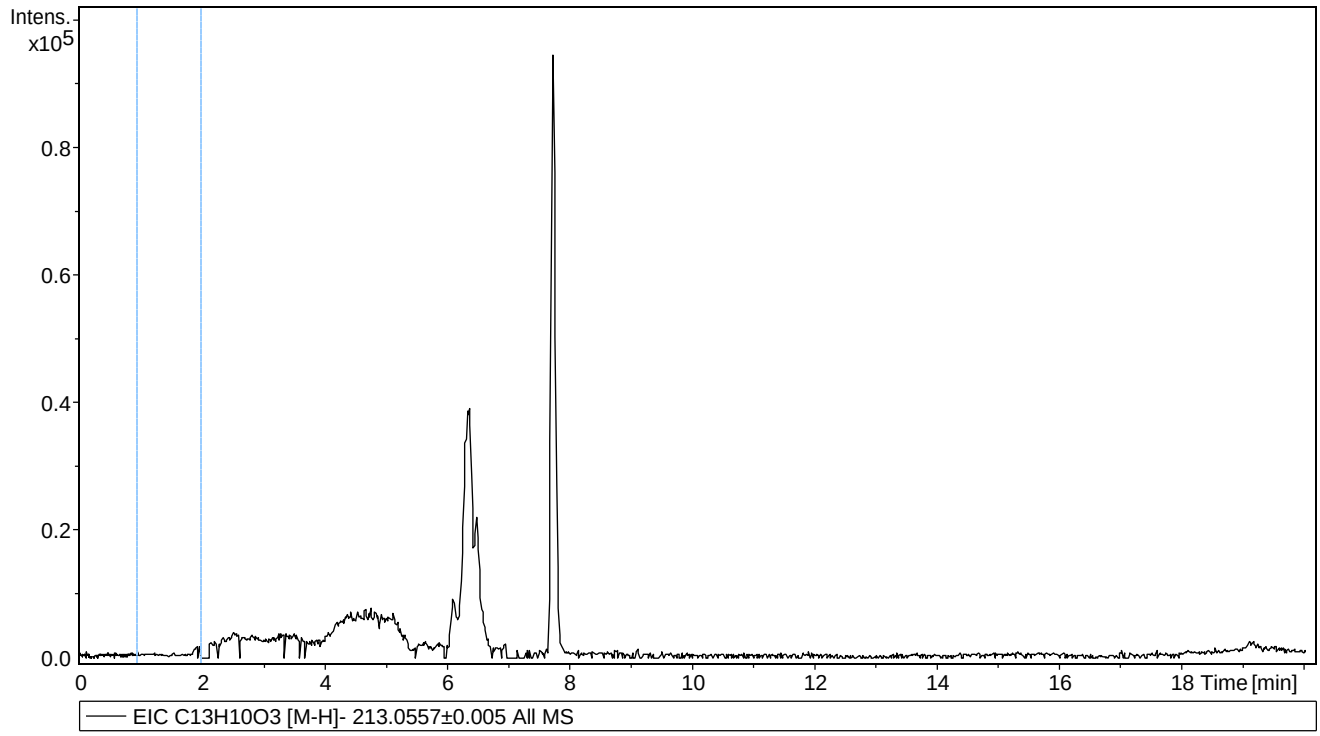
Operator BDAL@DE

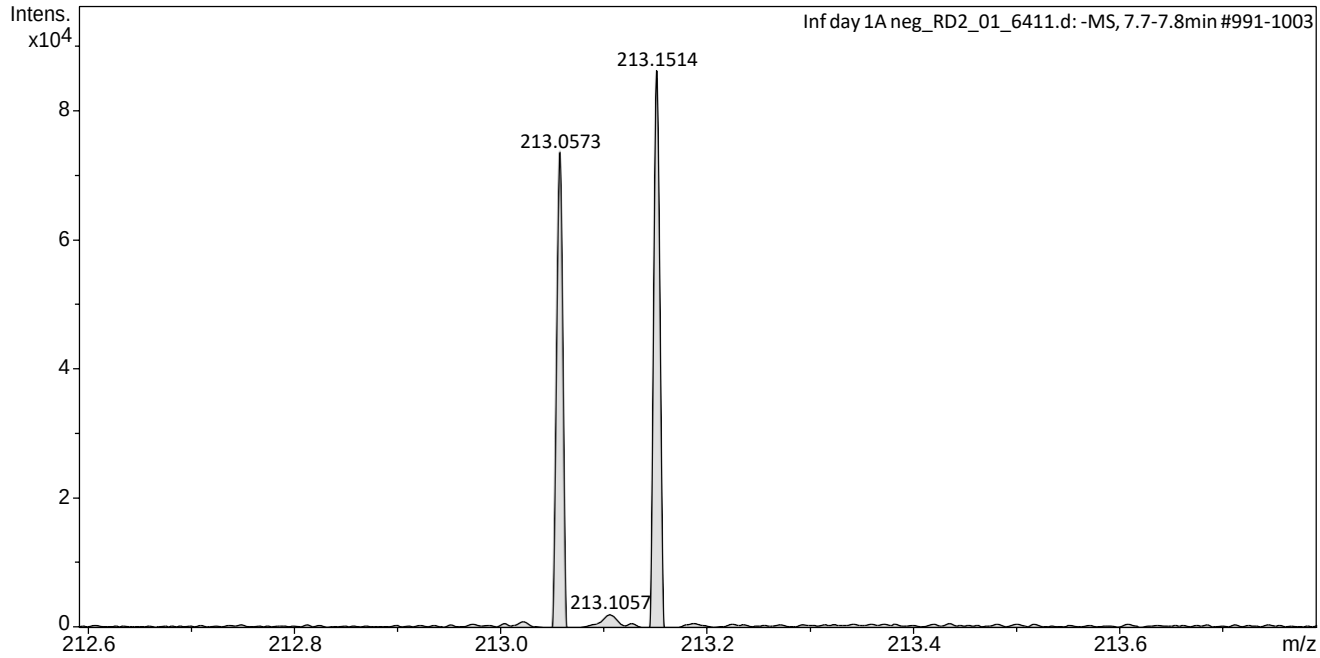
Sample Name Inf day 1A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6411.d

Display Report

Analysis Info

Acquisition Date 22/10/201607 :02:15

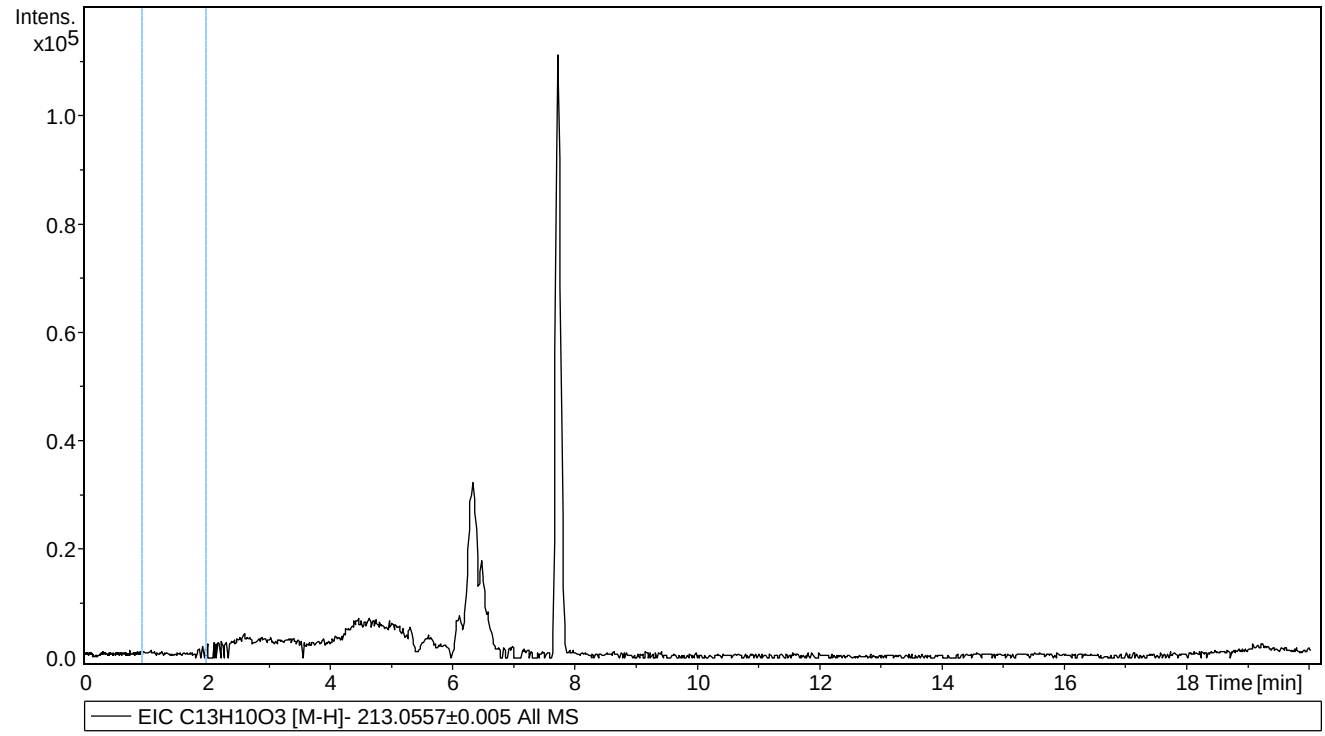
Operator BDAL@DE

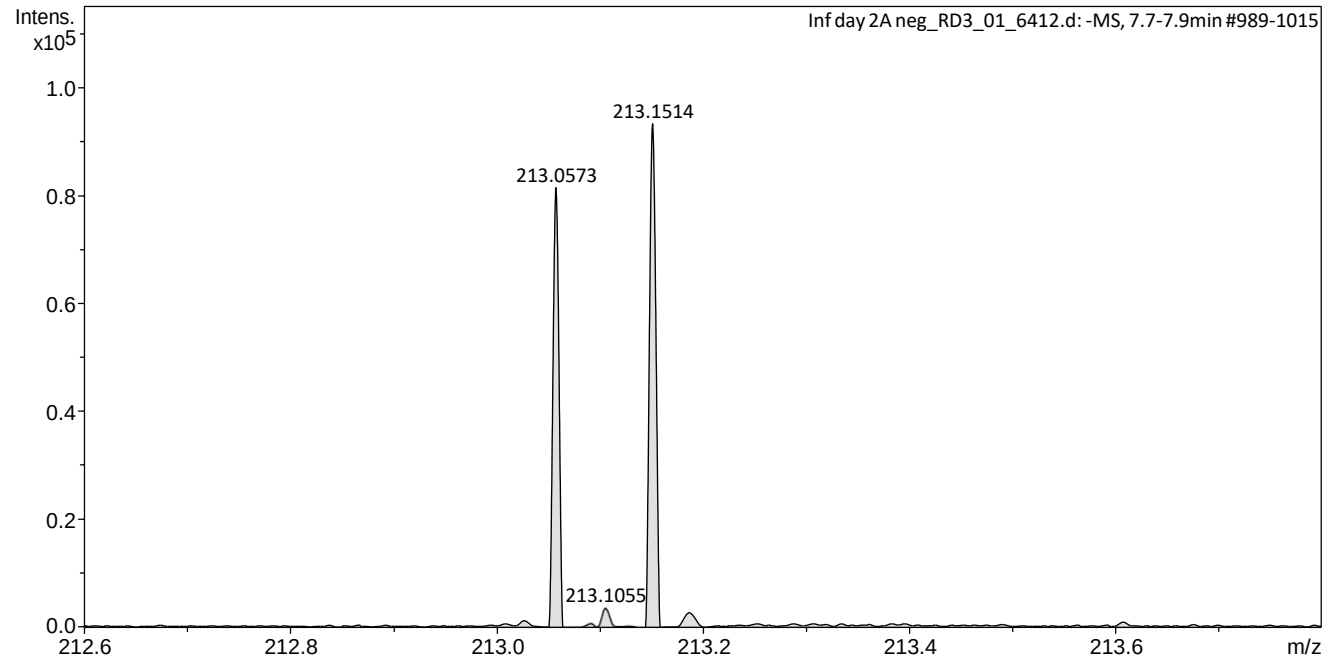
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 2A neg_RD3_01_6412.d

Display Report

Analysis Info

Acquisition Date 14/03/2017 21:08:18

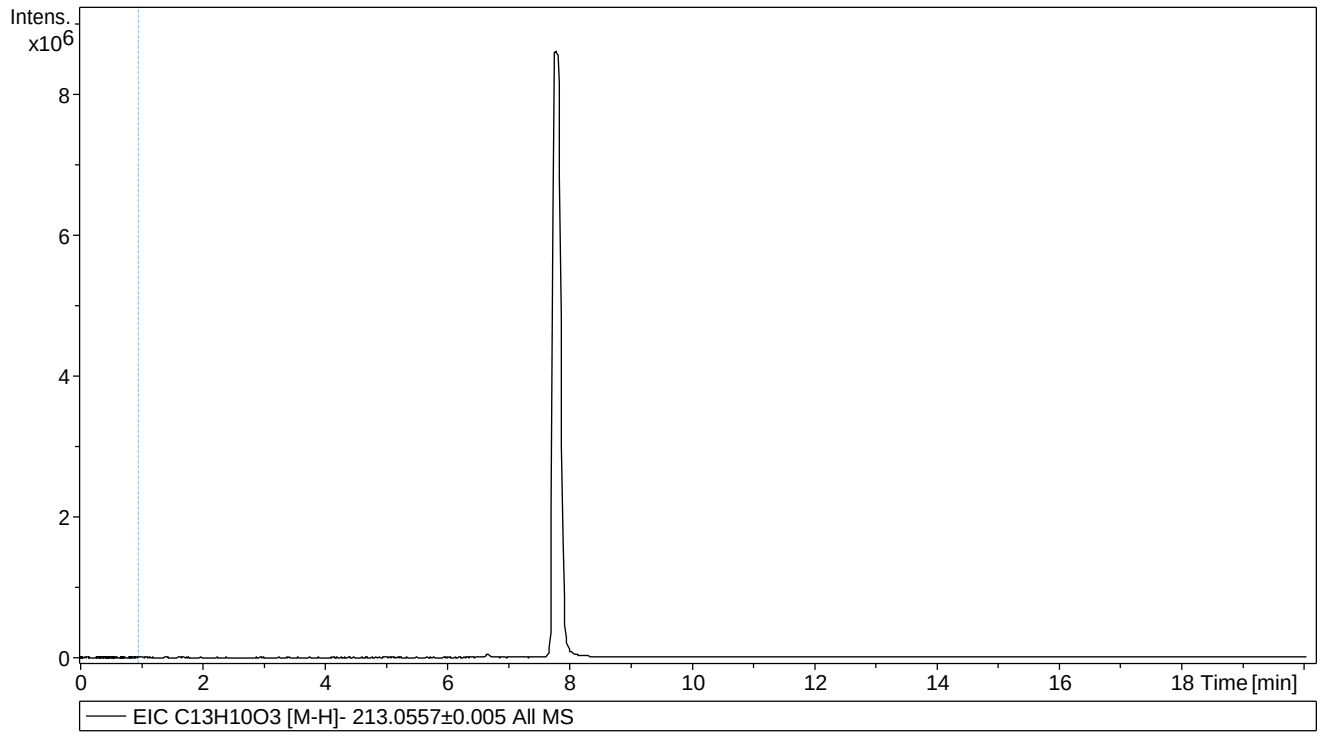
Operator BDAL@DE

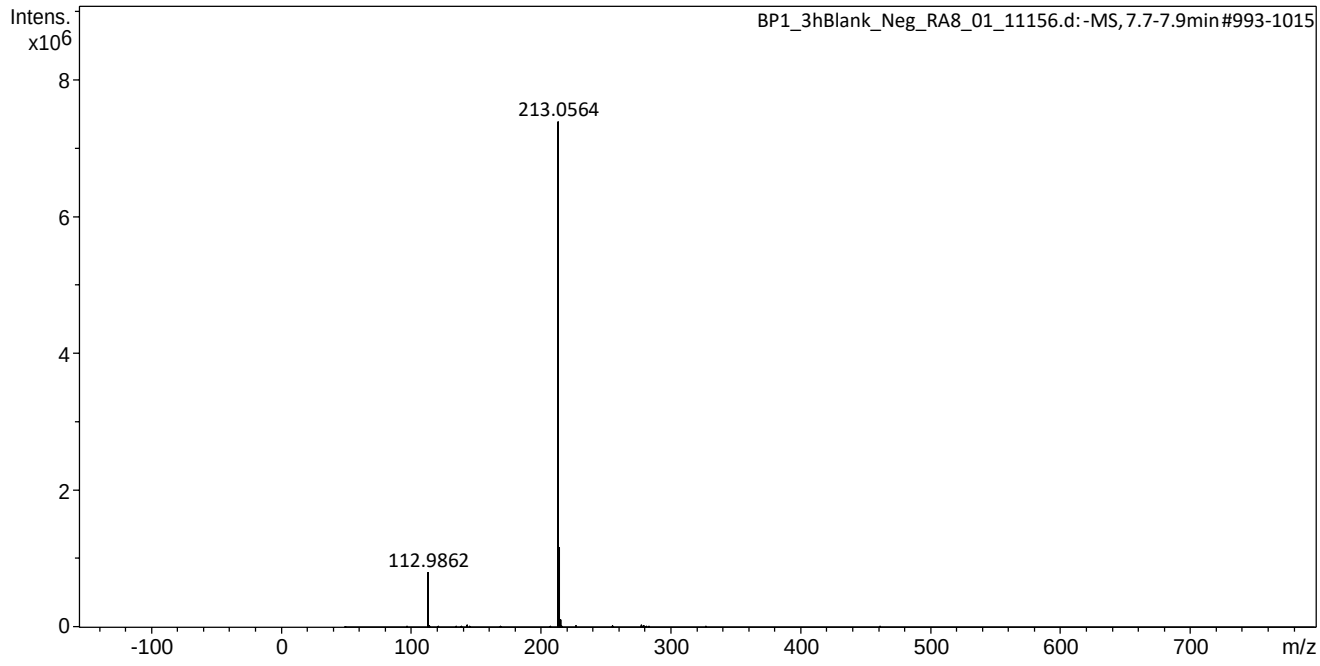
Sample Name BP1_3hBlank_Neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/10/2016 04:37:53

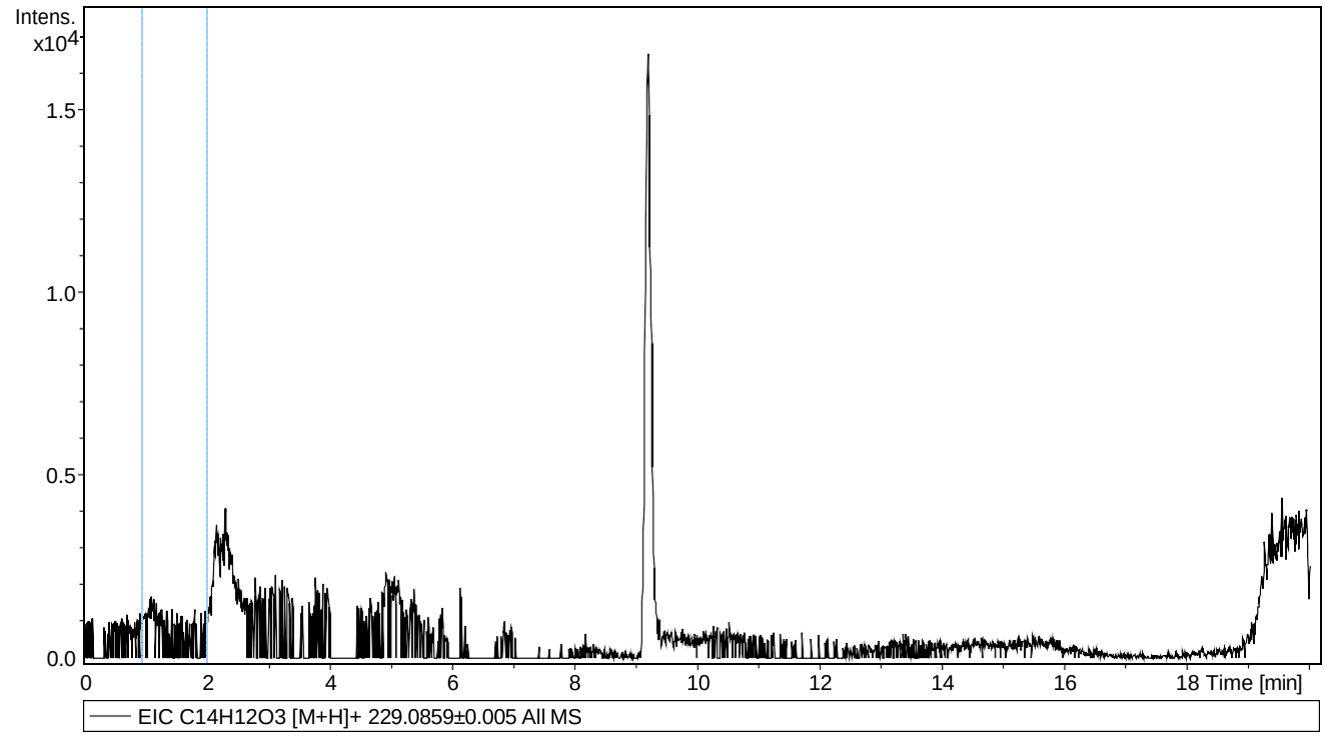
Operator BDAL@DE

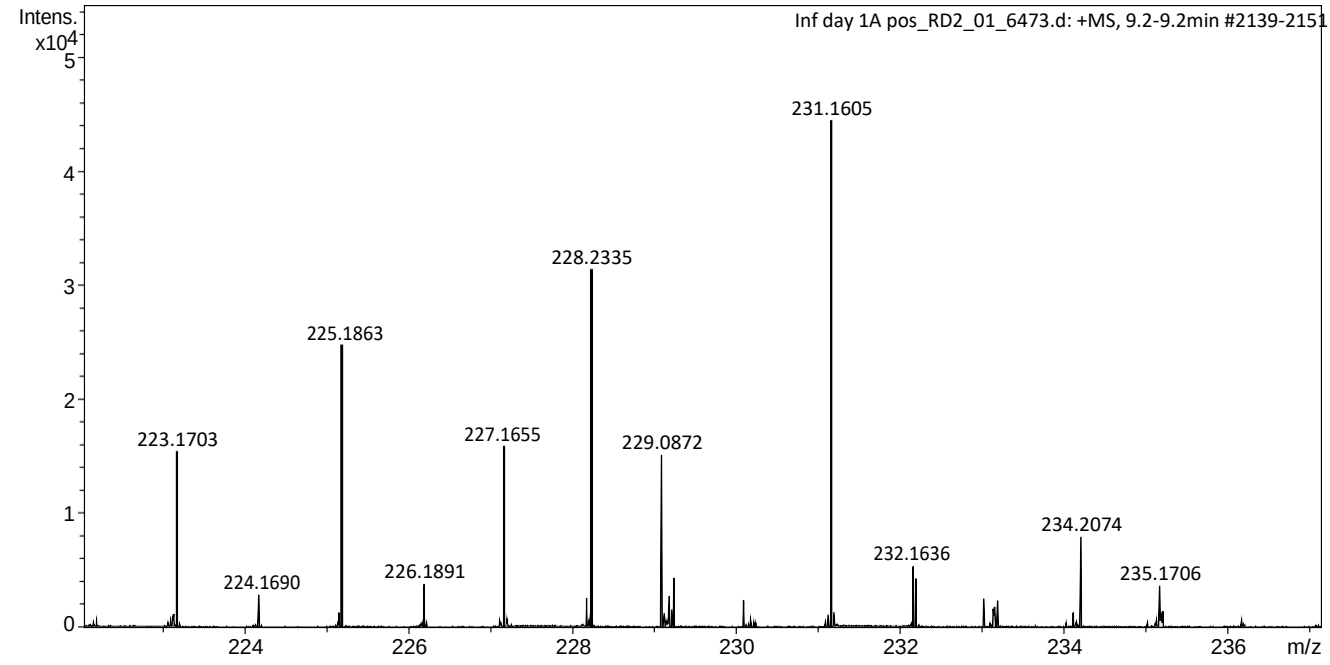
Sample Name Inf day 1A pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 23/10/2016 04:59:05

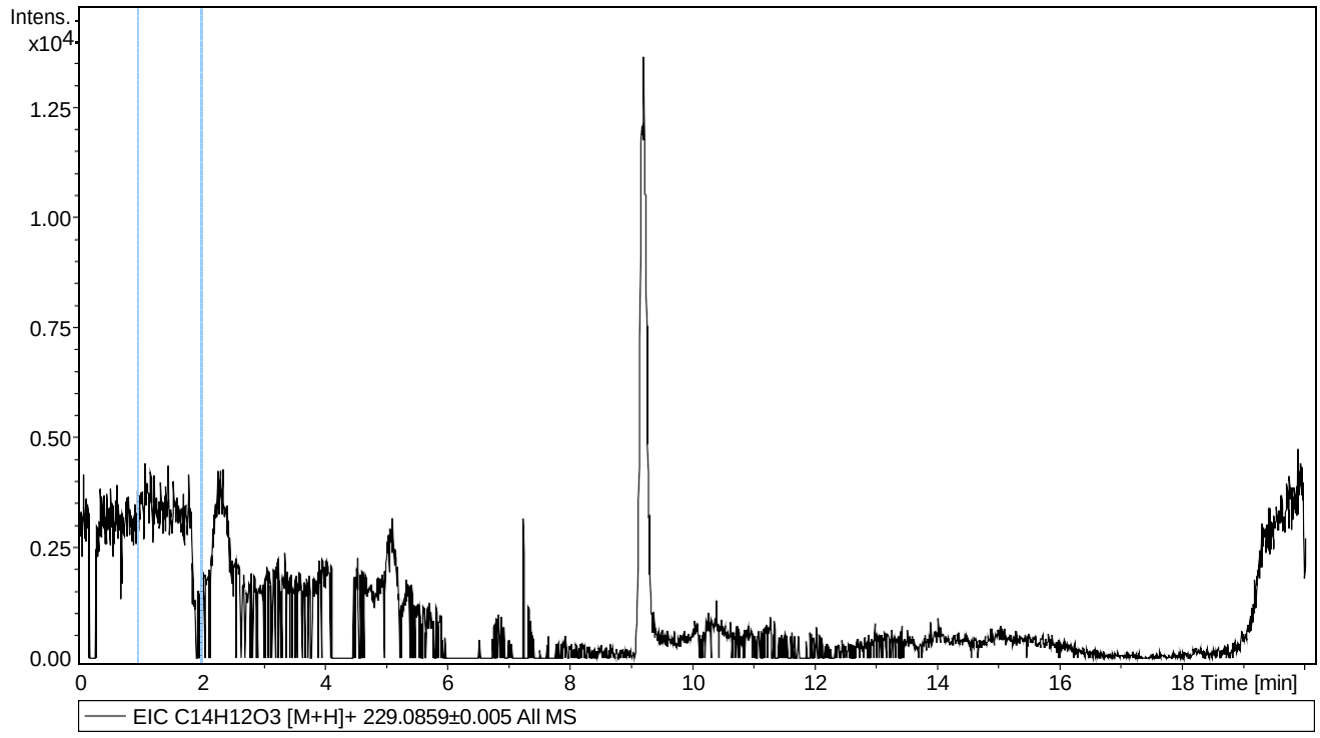
Operator BDAL@DE

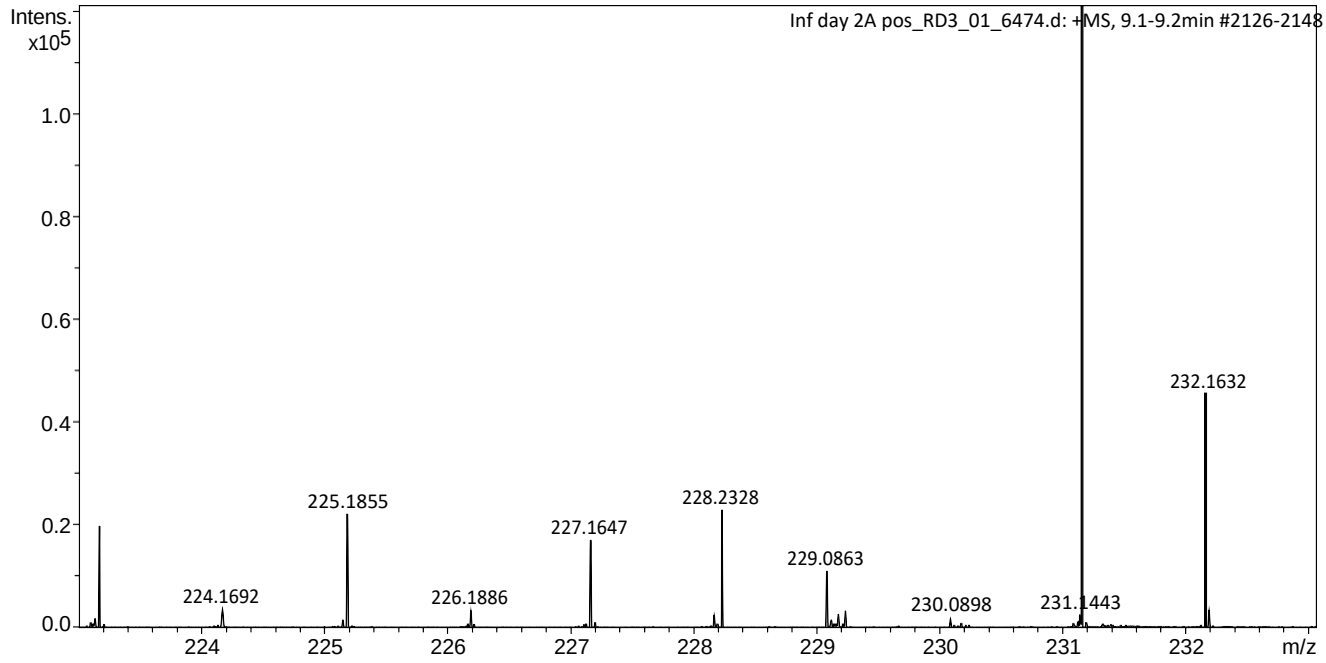
Sample Name Inf day 2A pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 24/11/2016 02:43:30

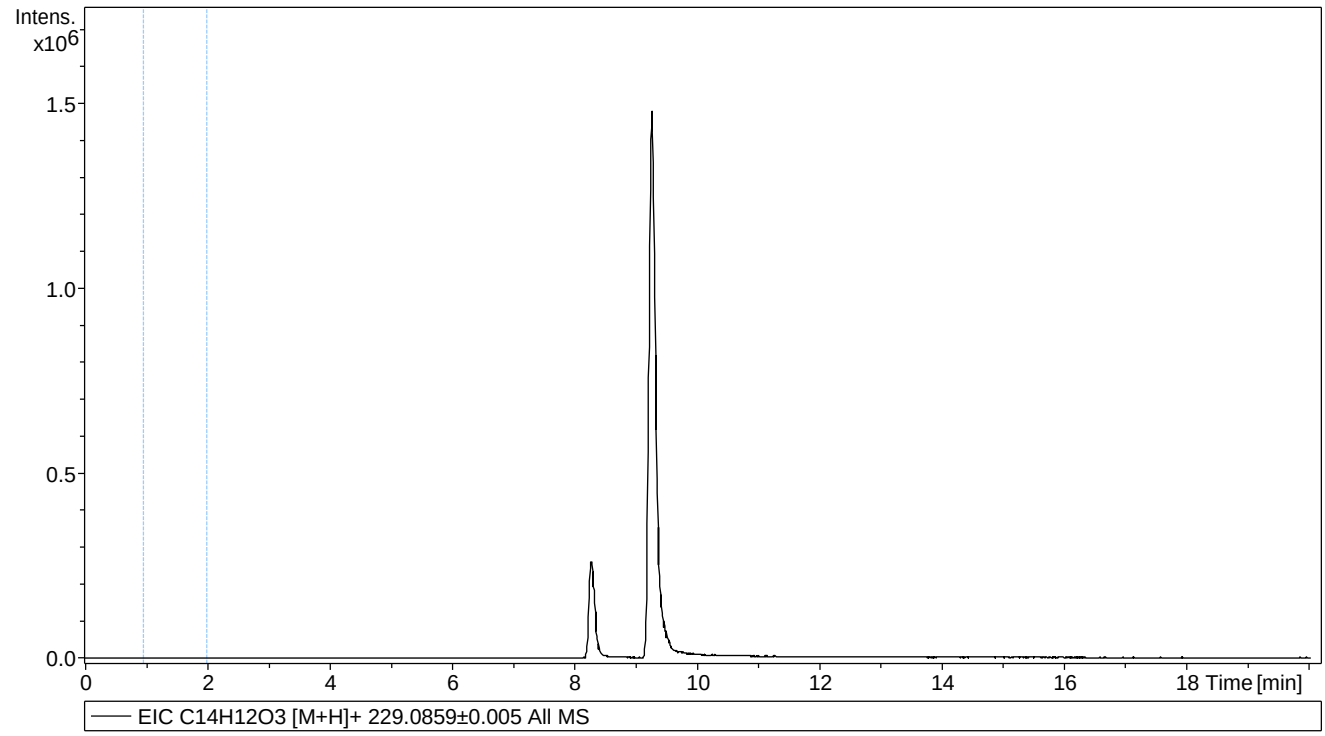
Operator BDAL@DE

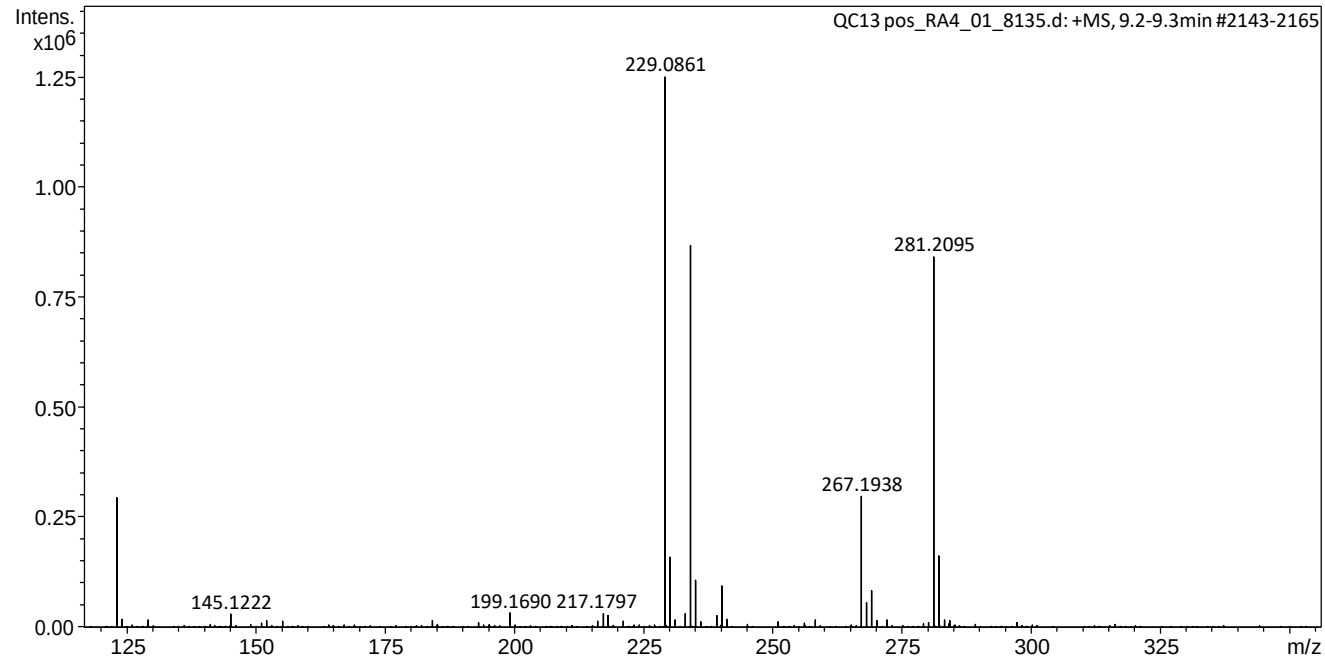
Sample Name QC13 pos

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

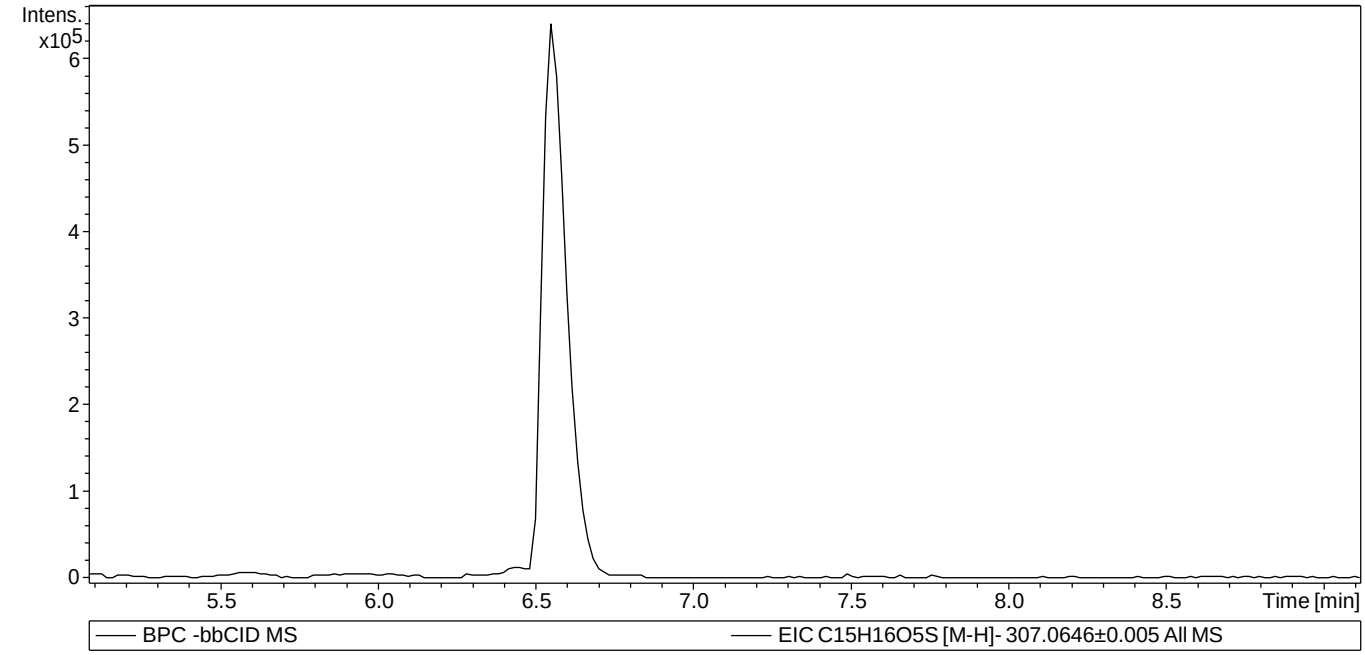
Acquisition Date 10/19/2016 8:26:24 PM

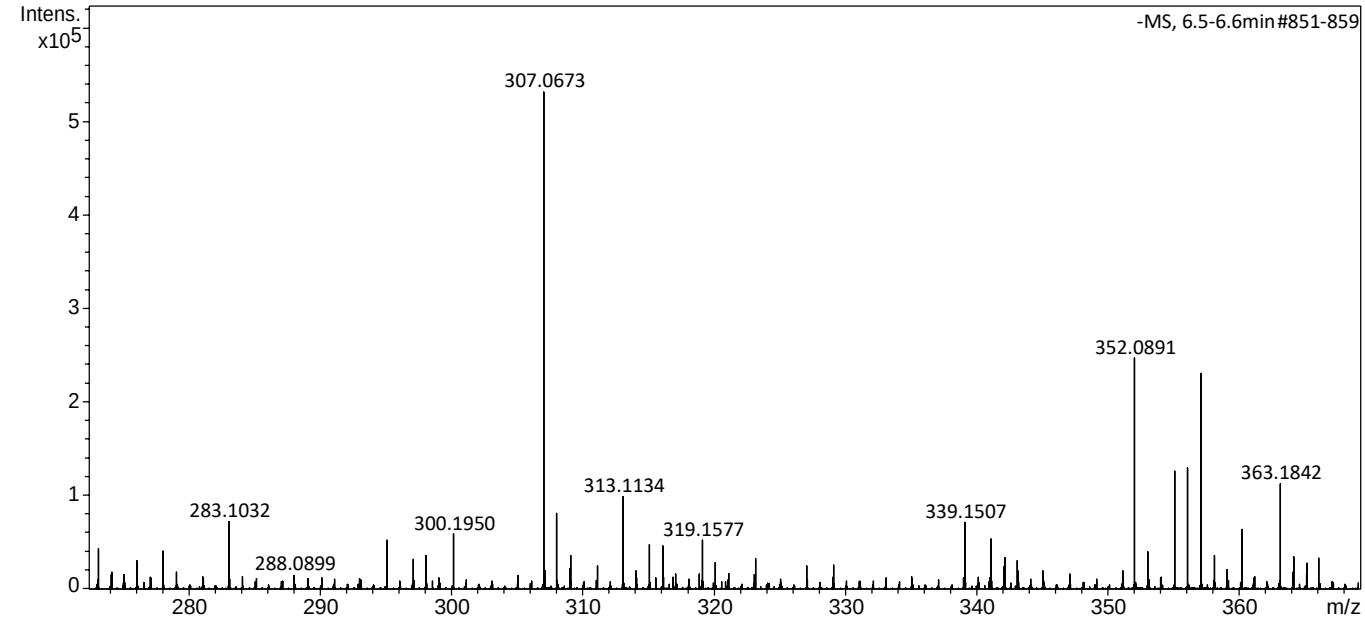
Sample Name Inf day 1A neg

Operator BDAL@DE
Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

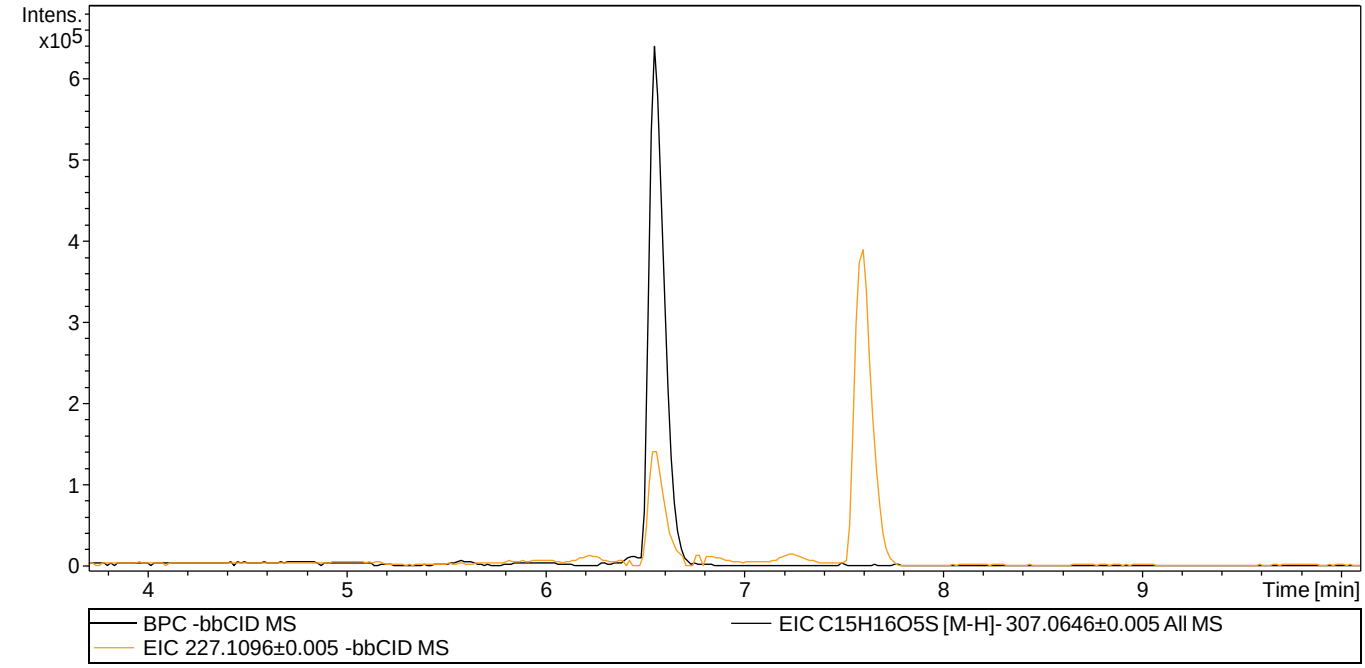
Display Report

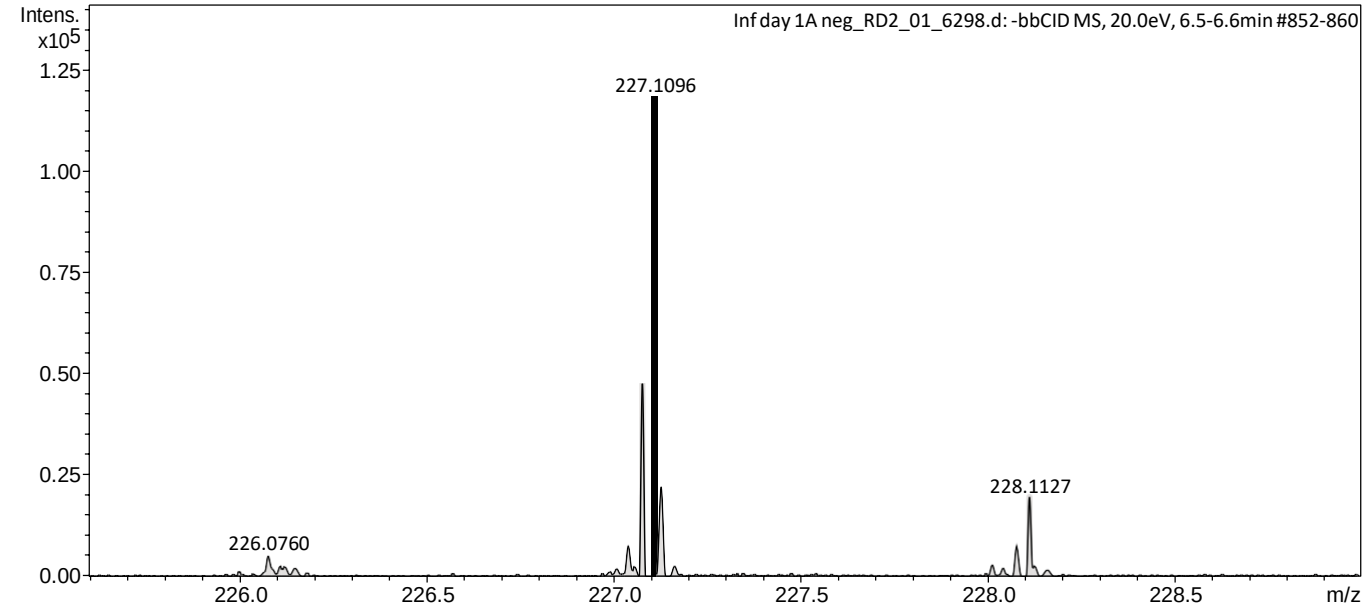
Analysis Info

Acquisition Date	10/19/2016 8:26:24 PM		
Sample Name	Inf day 1A neg	Operator	BDAL@DE
		Instrument	maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6298.d

Display Report

Analysis Info

Acquisition Date 19/10/2016 20:47:37

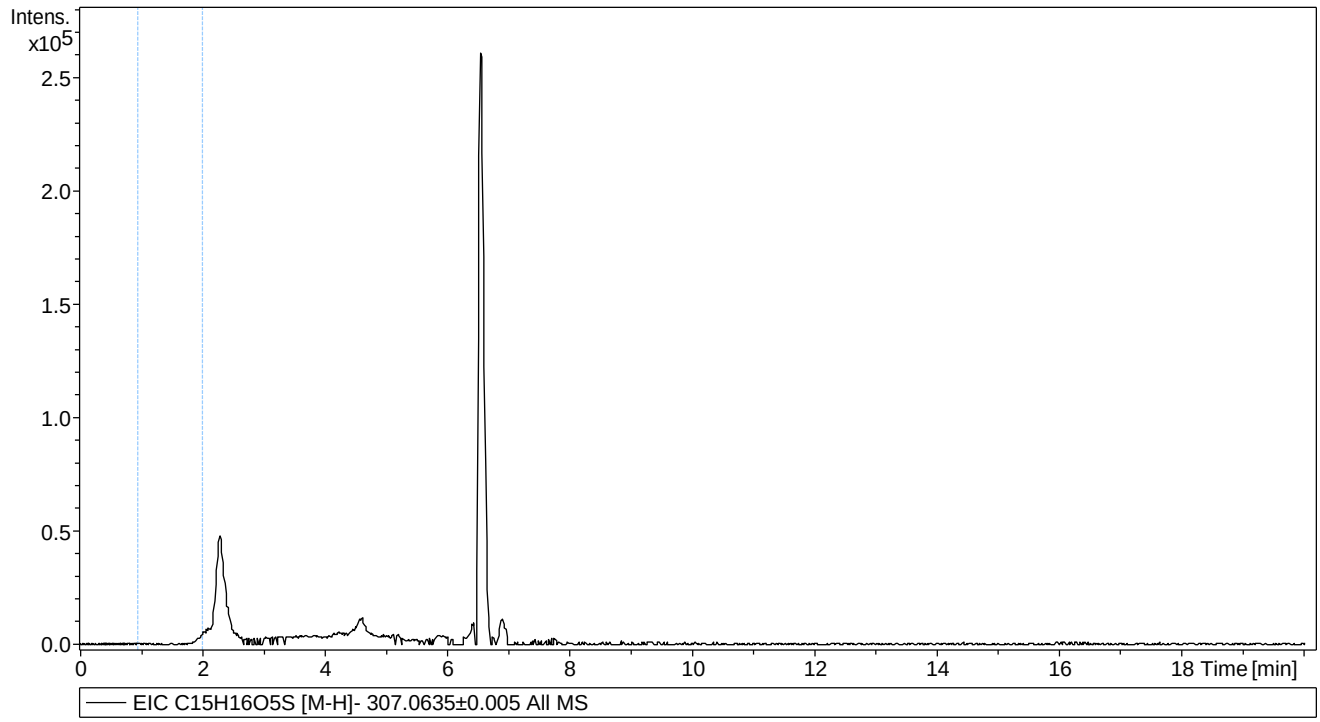
Operator BDAL@DE

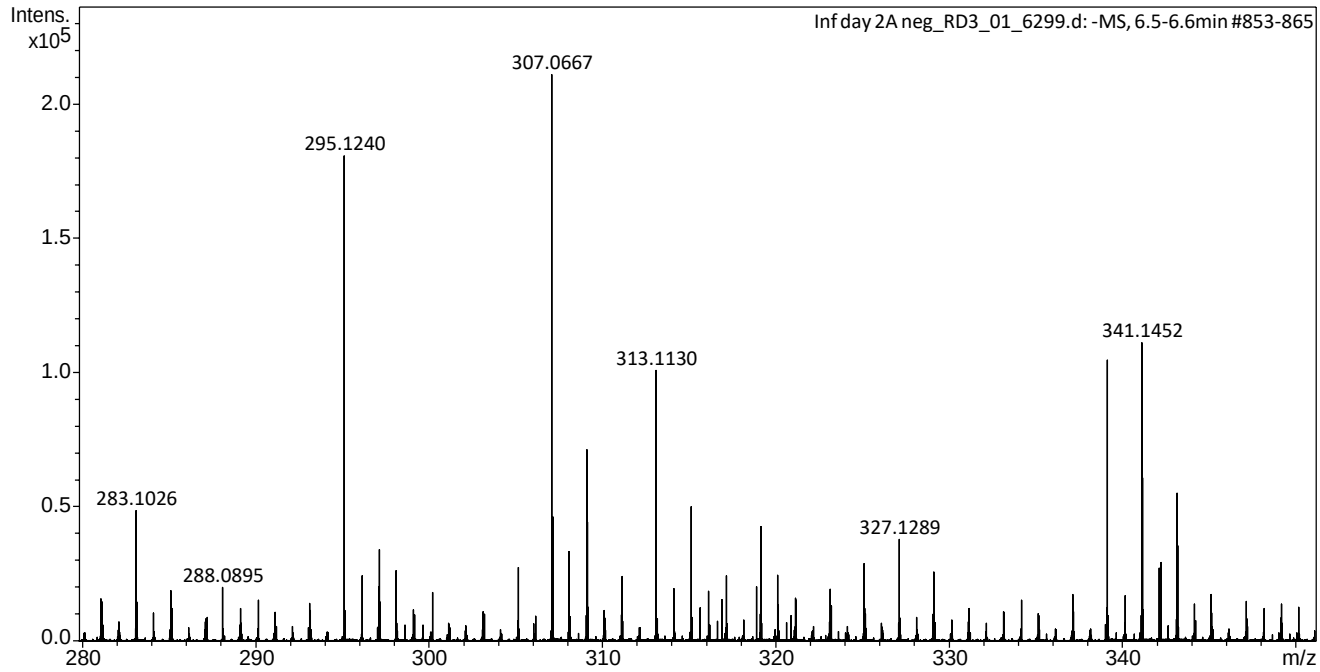
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 2A neg_RD3_01_6299.d

Display Report

Analysis Info

Acquisition Date 19/10/2016 20:47:37

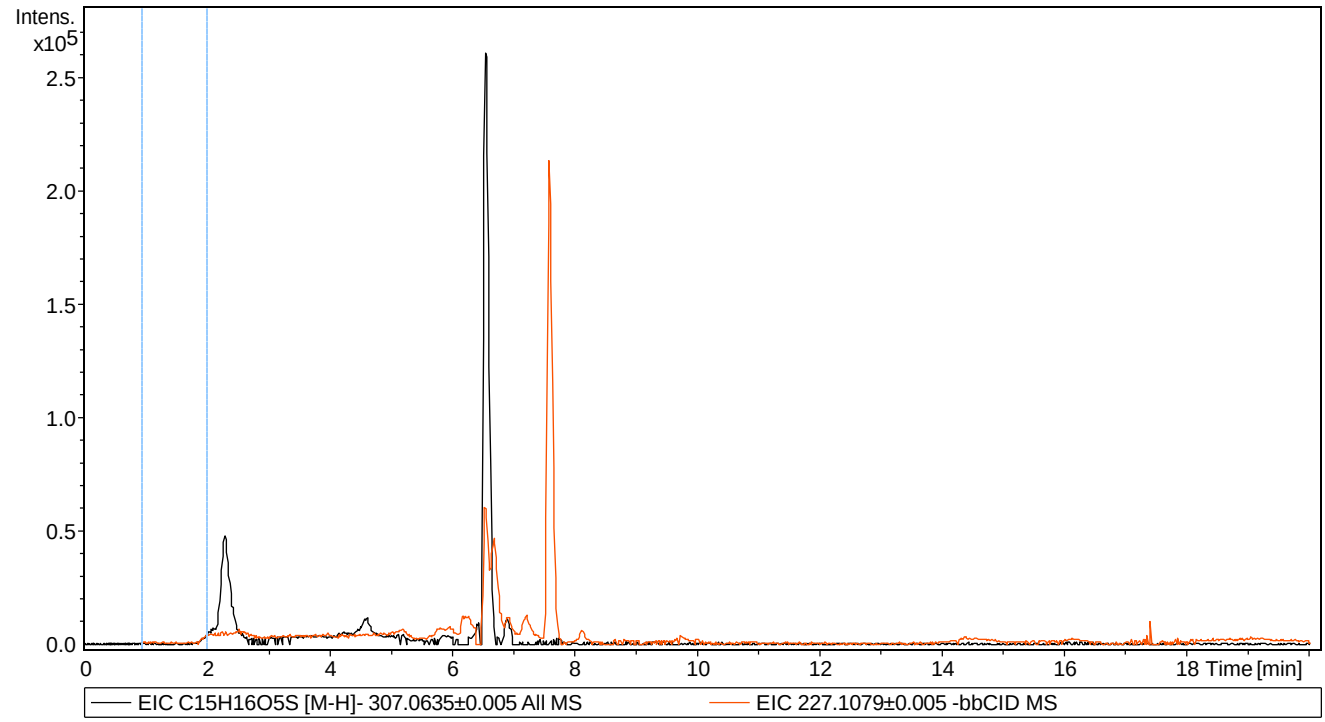
Operator BDAL@DE

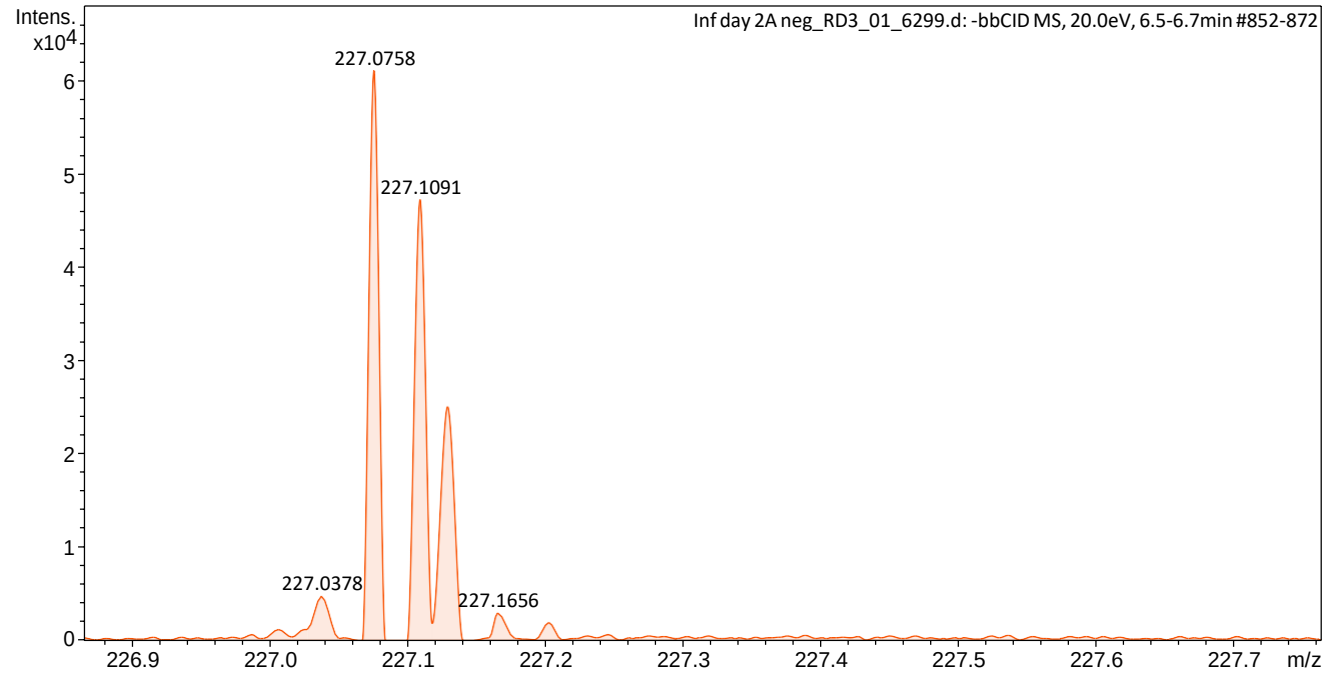
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 2A neg_RD3_01_6299.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 06:41:01

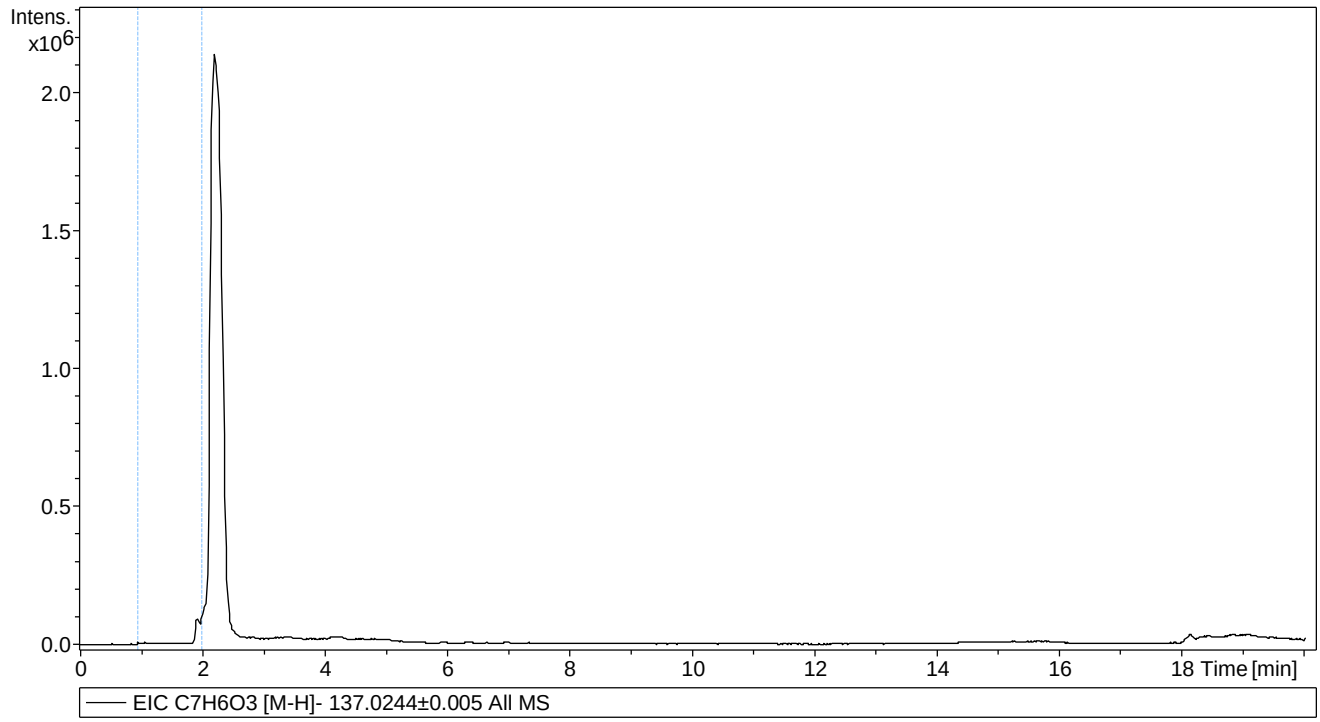
Operator BDAL@DE

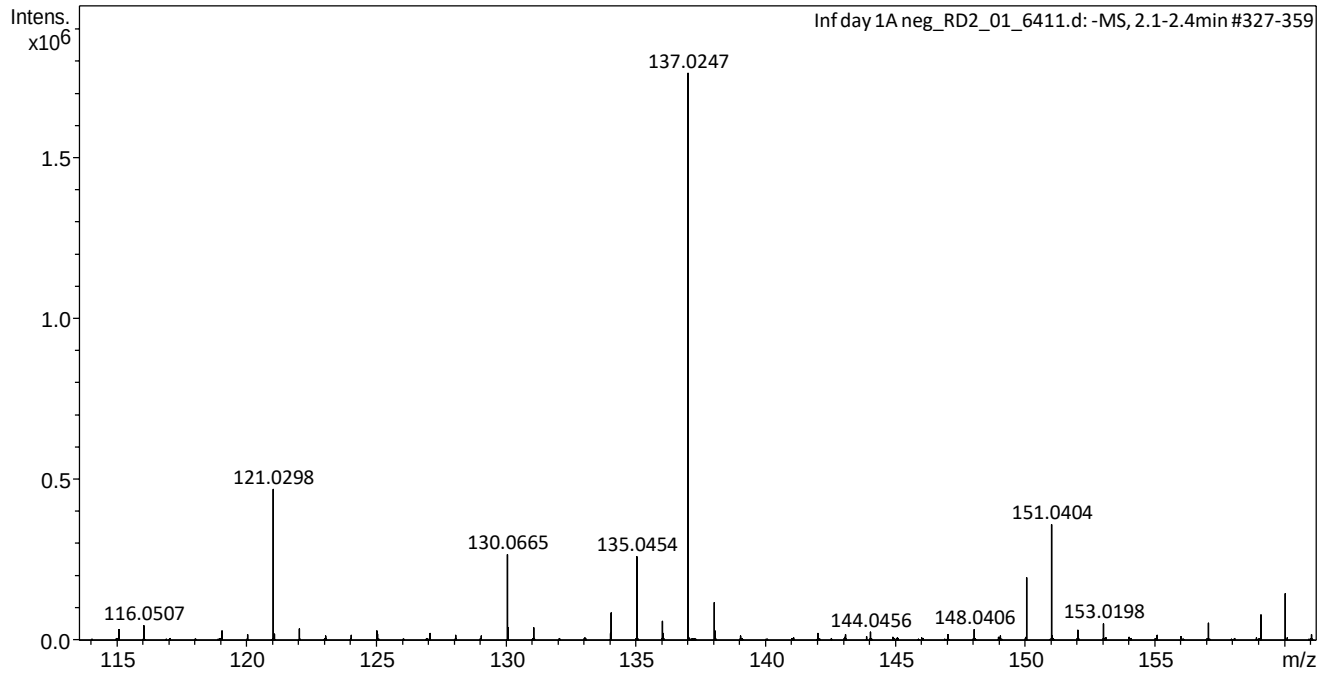
Sample Name Inf day 1A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6411.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 06:41:01

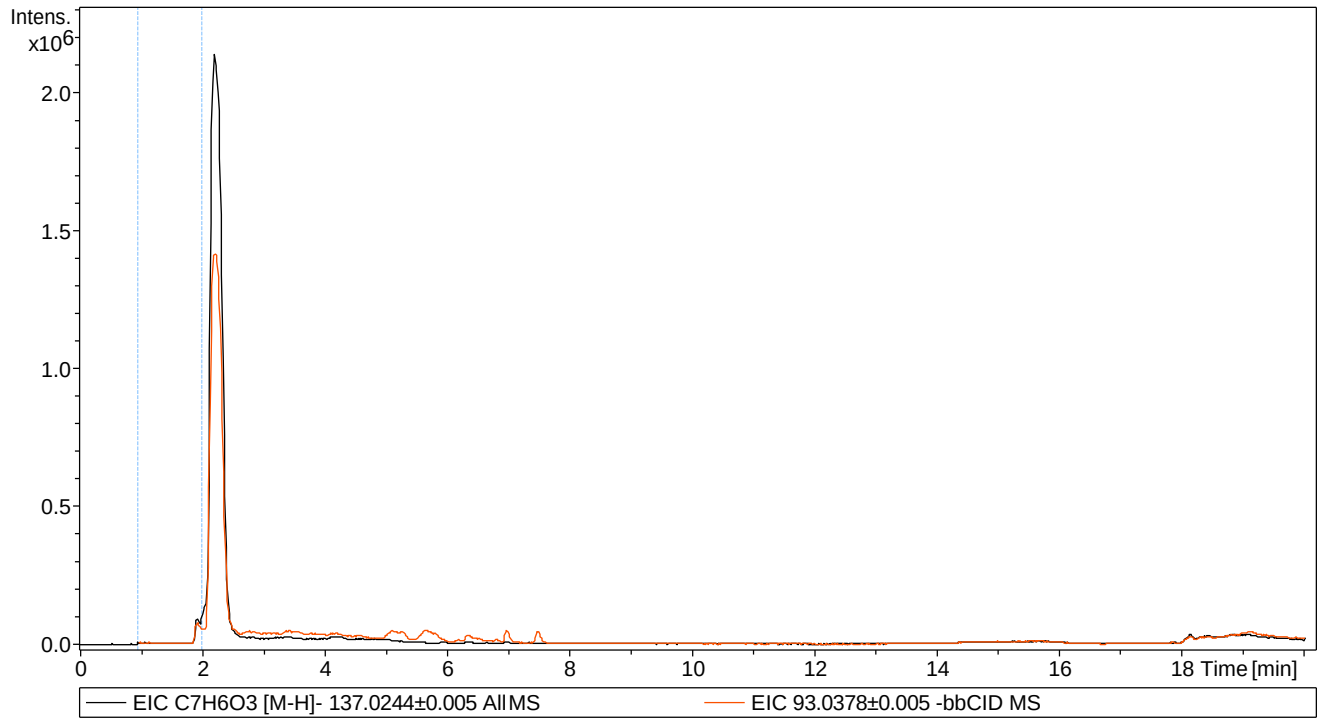
Operator BDAL@DE

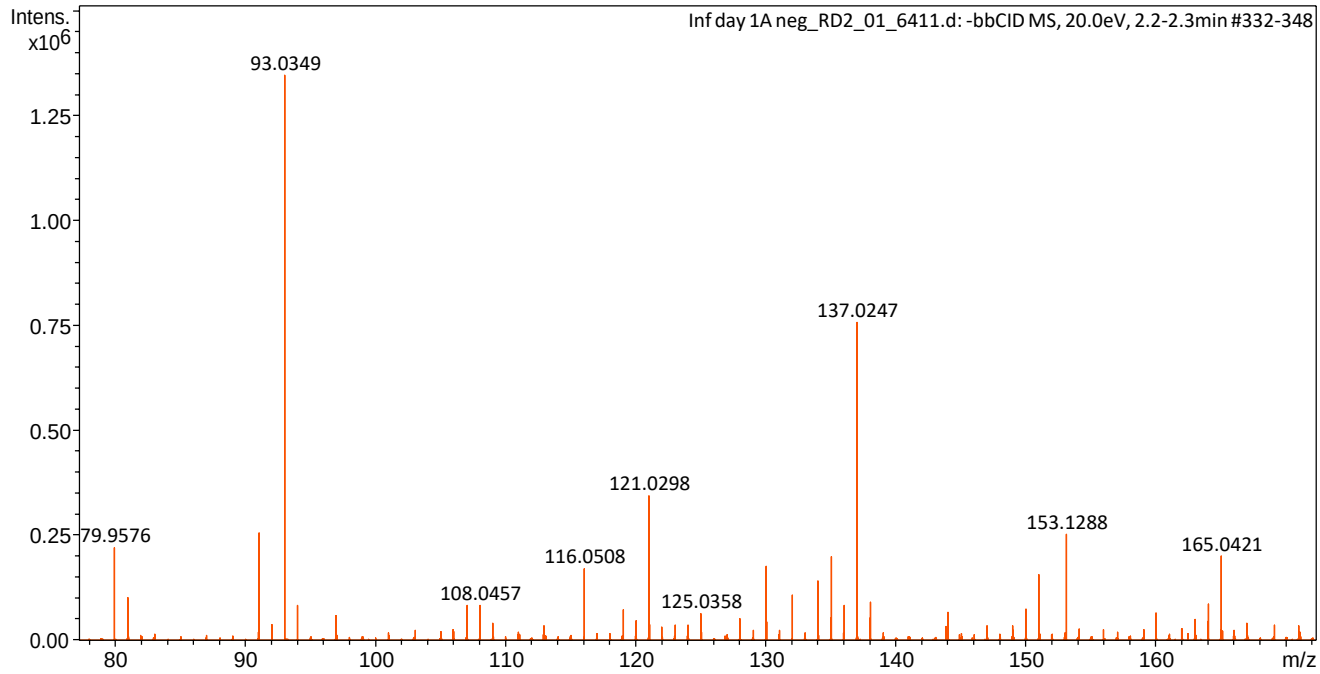
Sample Name Inf day 1A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6411.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 07:02:15

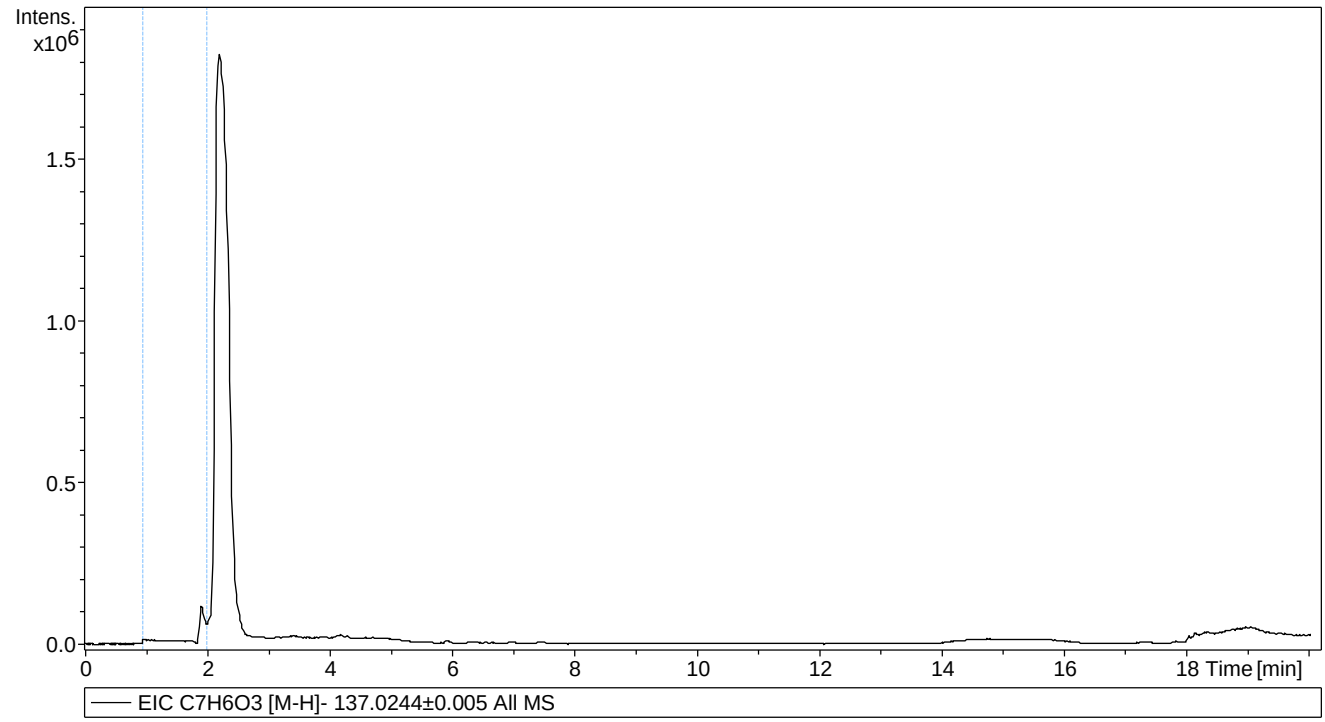
Operator BDAL@DE

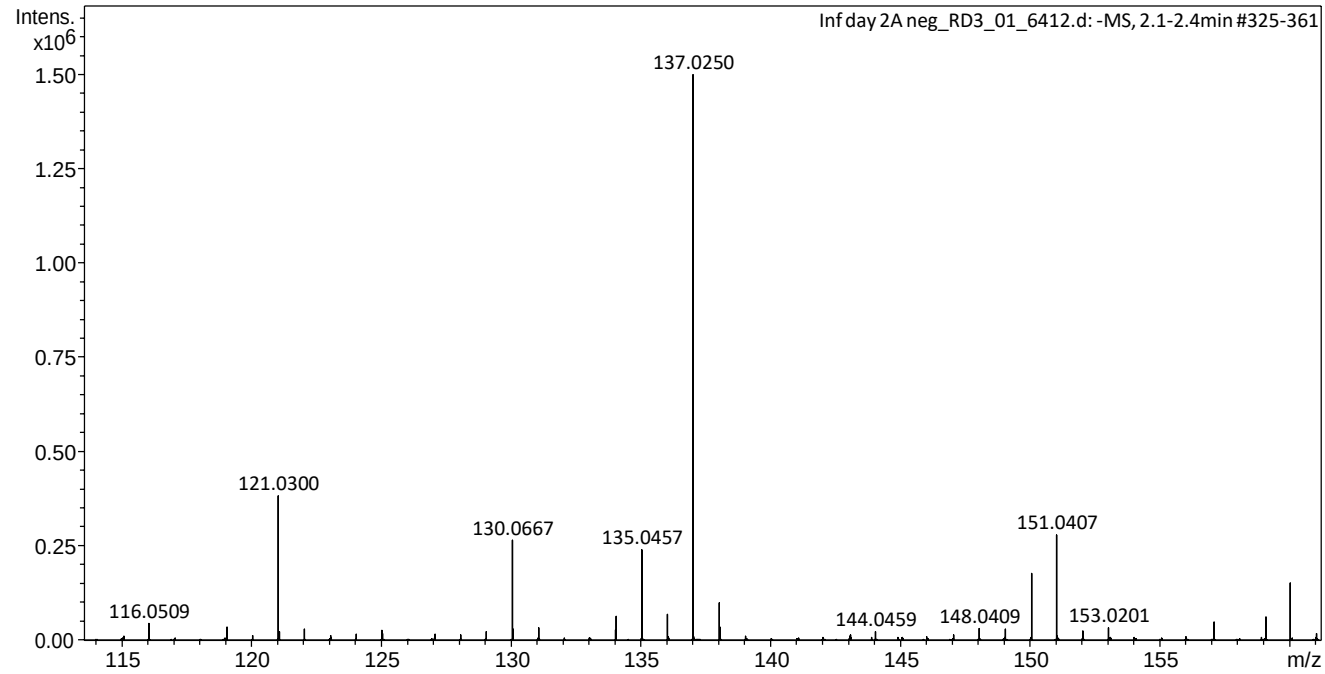
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 22/10/2016 07:02:15

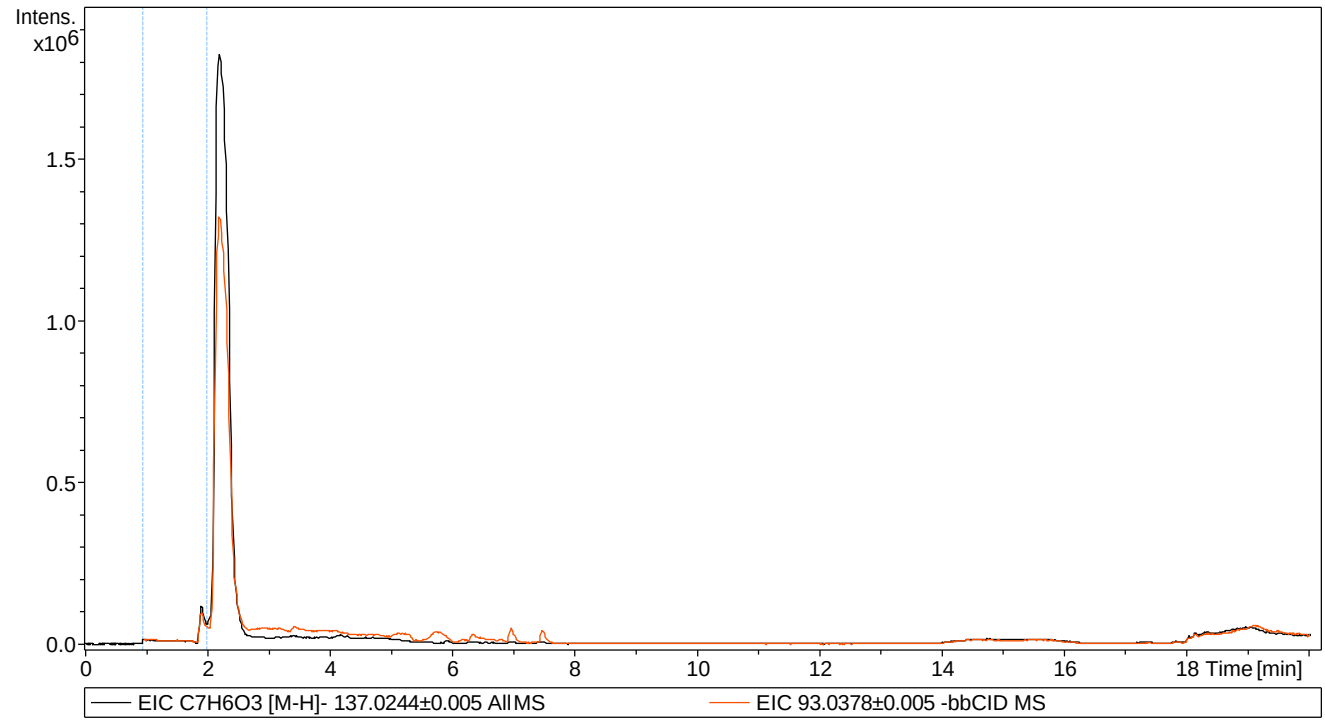
Operator BDAL@DE

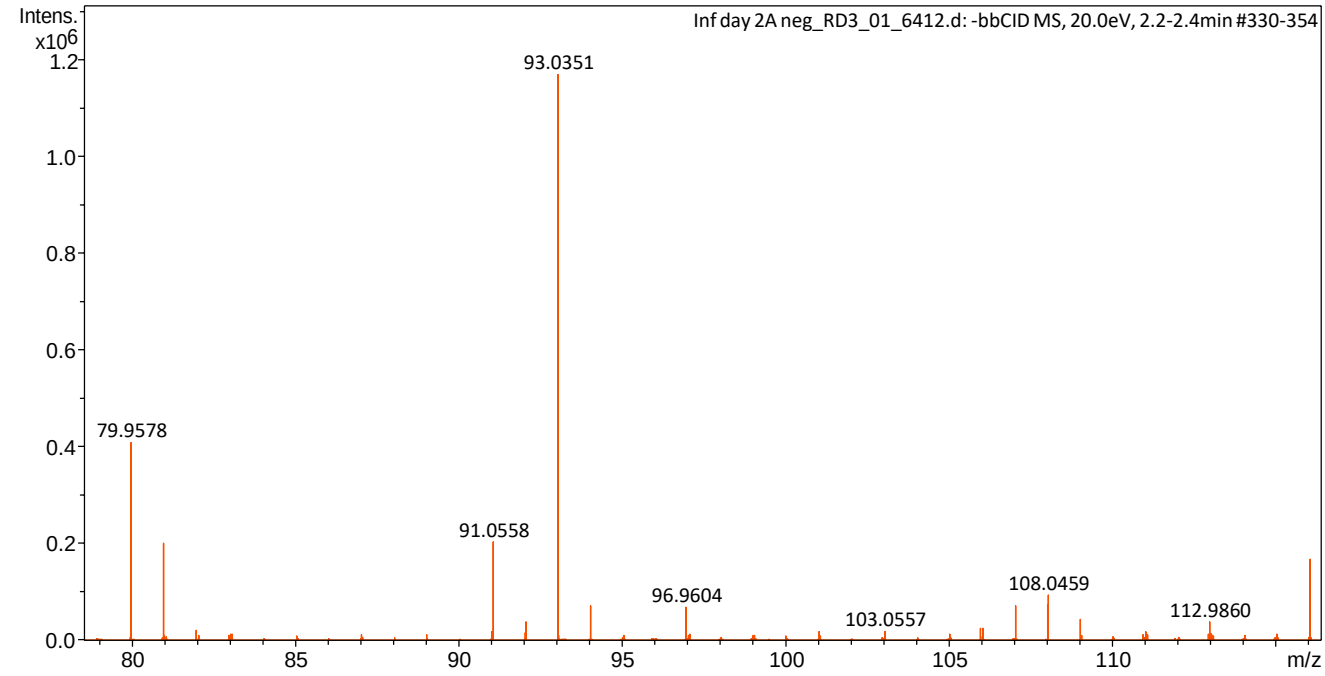
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 2A neg_RD3_01_6412.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 06:41:01

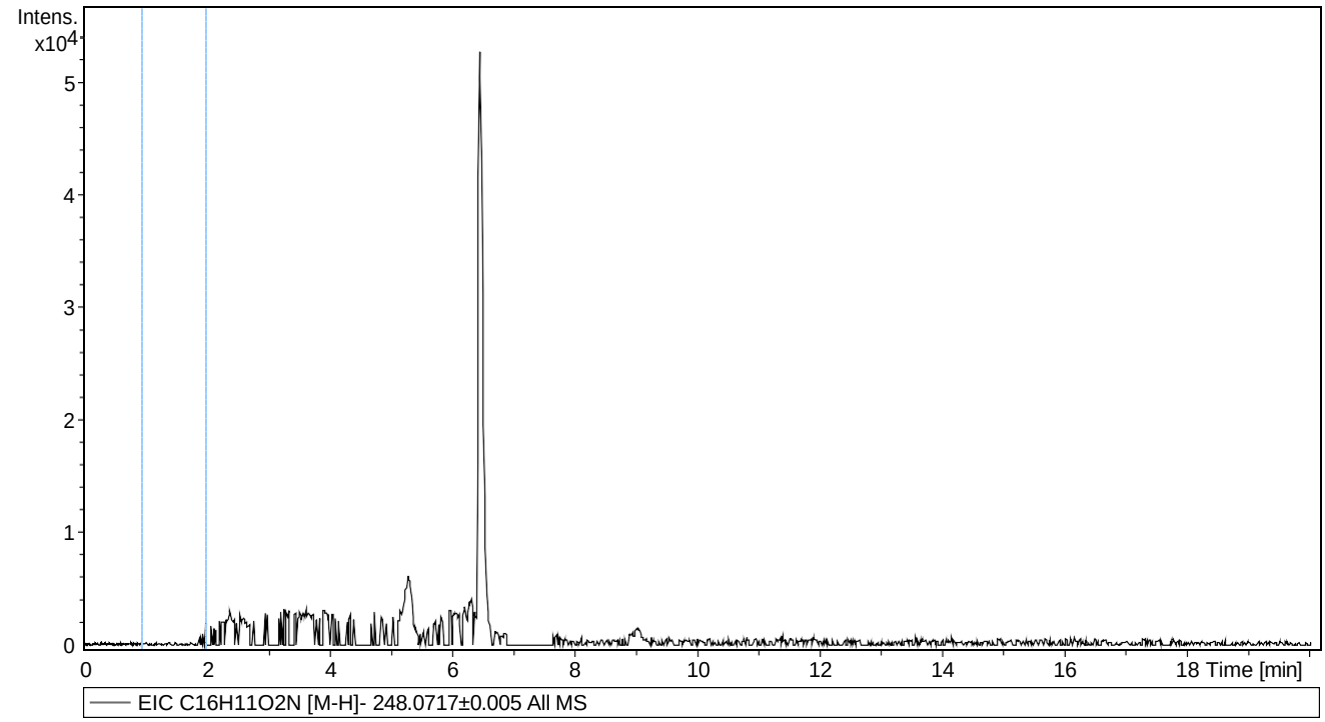
Operator BDAL@DE

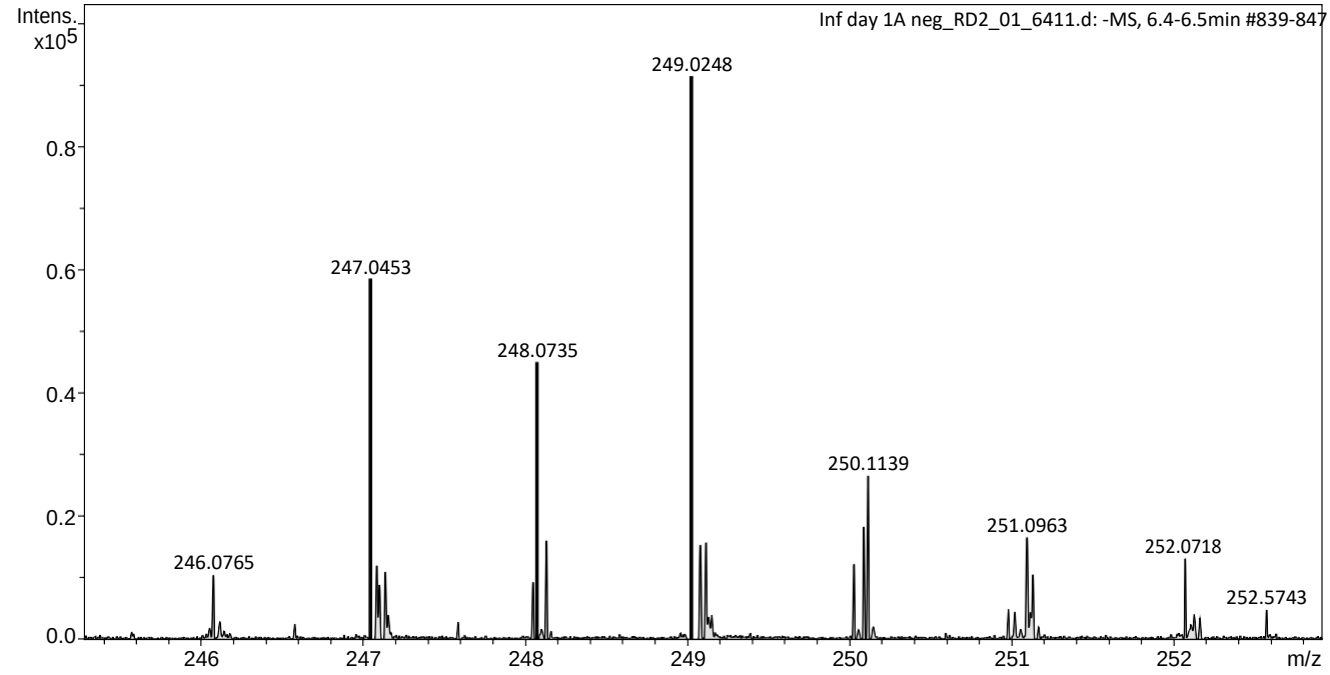
Sample Name Inf day 1A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Display Report

Analysis Info

Acquisition Date 22/10/2016 06:41:01

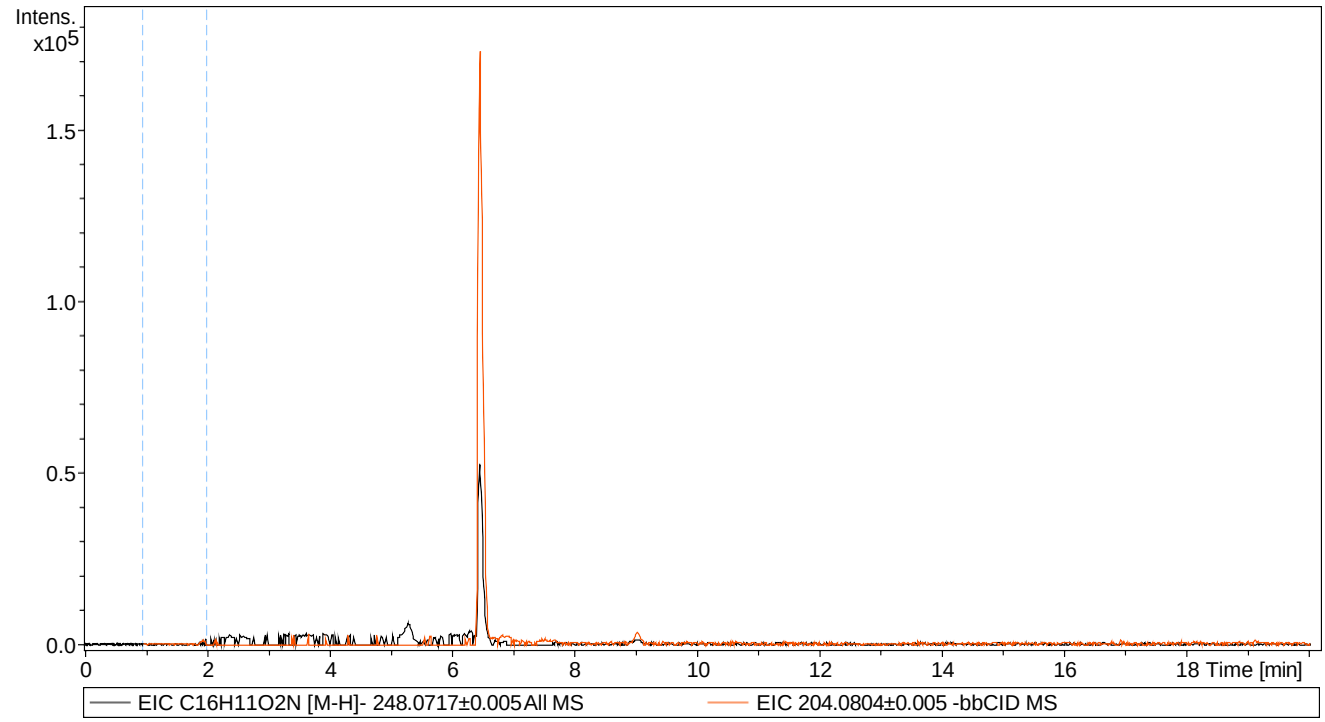
Operator BDAL@DE

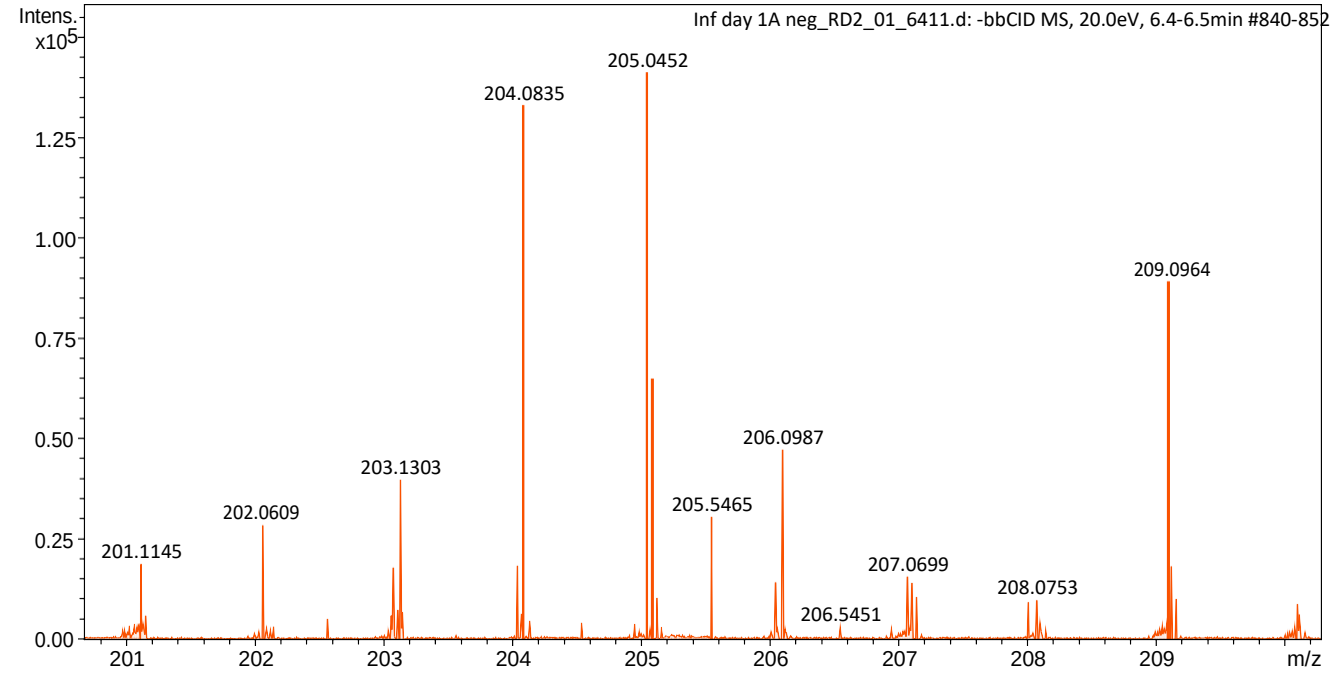
Sample Name Inf day 1A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 1A neg_RD2_01_6411.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 07:02:15

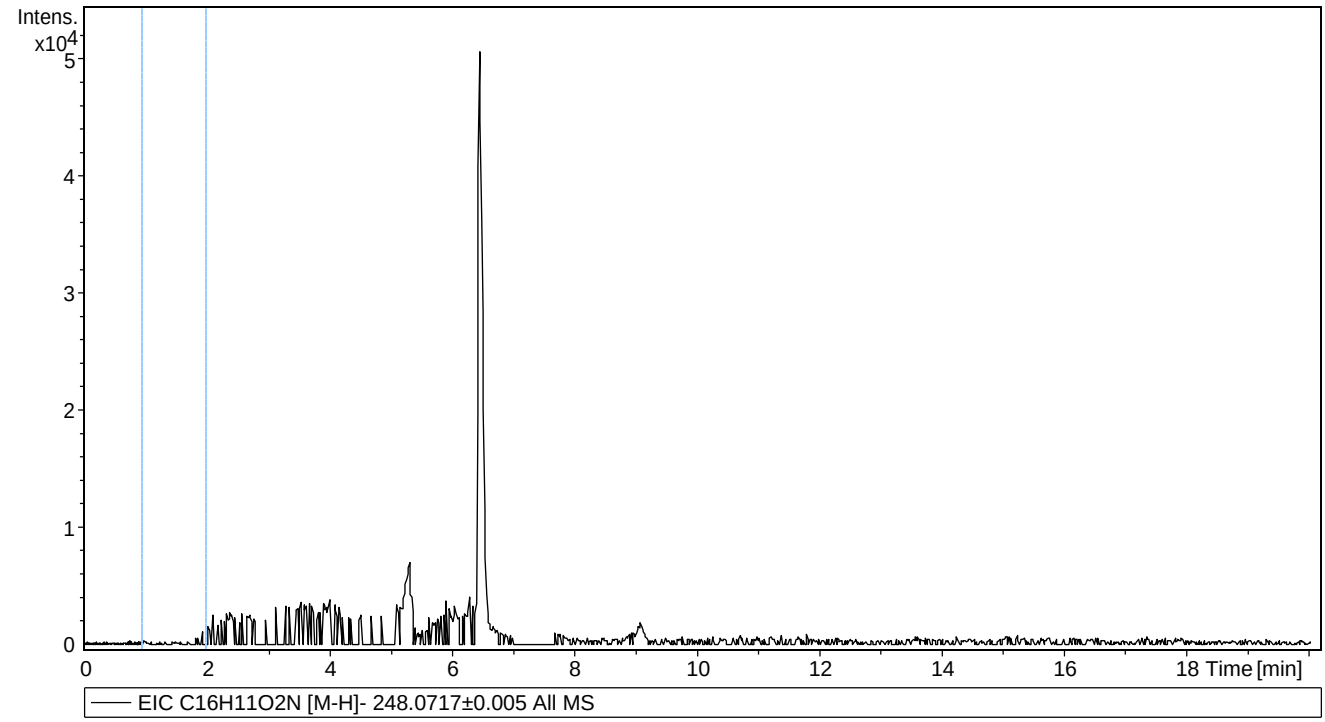
Operator BDAL@DE

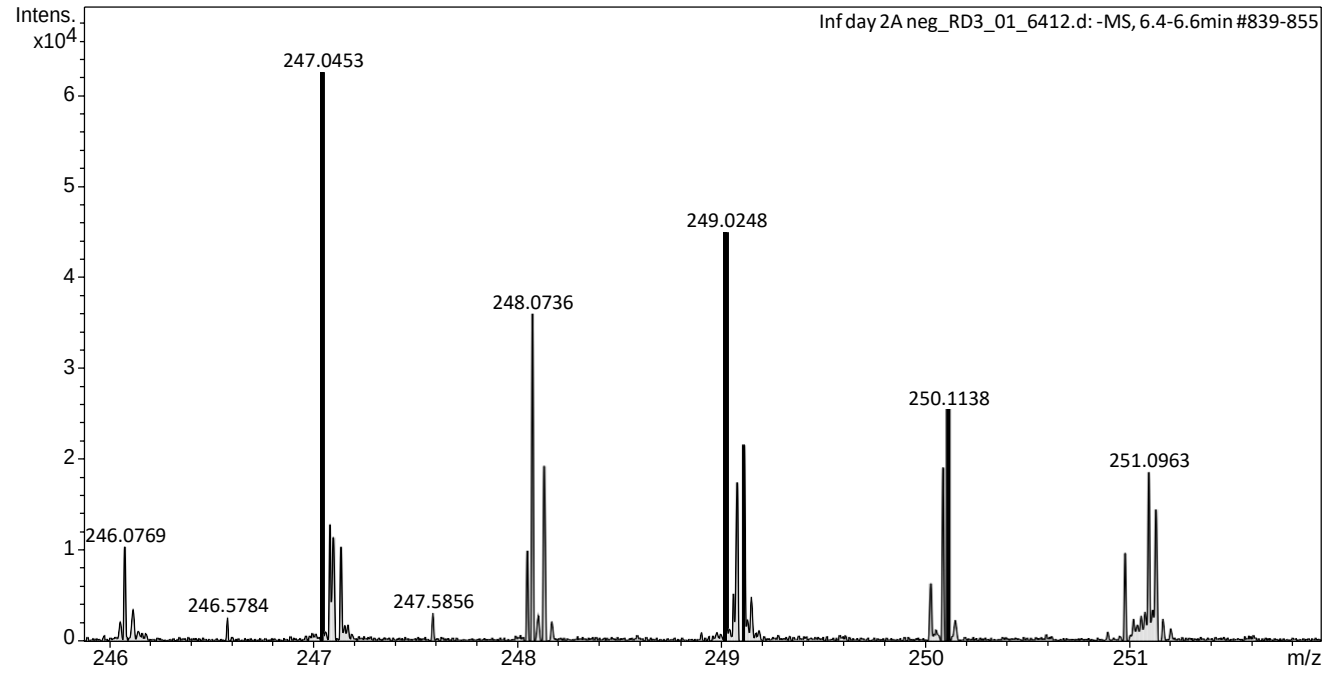
Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C





Inf day 2A neg_RD3_01_6412.d

Display Report

Analysis Info

Acquisition Date 22/10/2016 07:02:15

Operator BDAL@DE

Sample Name Inf day 2A neg

Instrument maXis-HD 1820881.21247

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	3.0 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	11.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	4000 nA	Set APCI Heater	200 °C